AUGMENTED CELL ADHESION OF REGENERATIVE HEPATIC PROGENITORS VIA CLICK-MEDIATED SURFACE RECRUITMENT OF MACROMOLECULAR BIOPOLYMERS.

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Abstract

The optimisation of cellular adhesion and homing for cell-based therapies remains an ongoing area of research as an avenue for increasing therapeutic efficacy. Cell-based therapies for liver diseases, for example, are hindered by sub-optimal efficacy due to limited cellular engraftment and viability *in vivo*. [1] Genetic modification (GM) of therapeutic cell candidates remains as a prevalent technique in this area. Indeed, it has been shown that the genetic overexpression of hyaluronic acid (HA) synthase isoforms can result in significant increases in mesenchymal stem cell adhesion. [2] Non-GM based surface functionalisation methods such as electrostatic surface deposition and amine-targeted covalent ligation aim to bypass the high costs and adverse mutative risks of GM but suffer from poor cytocompatibility and limited surface functionalisation and homogeneity. [3]

Metabolic oligosaccharide engineering (MOE) and strain promoted alkyne-azide (click) cycloaddition has emerged as a superior alternative to these approaches for the recruitment of diverse click-labile molecules to the cell surface in specific, bio-orthogonal, and highly efficient reactions. [4] Here, we propose the use of MOE for the covalent conjugation of exogenous materials to hepatic progenitor cell (HPC) membranes, previously highlighted in the literature as a potent but low-engrafting candidate for hepatic regeneration. [1,5]

Using MOE, we have developed a reproducible method for achieving homogenous surface coatings with functionalised macromolecules, achieving high cytocompatibility, with a transient residence time of < 48 h on the cell surface. Within 1 h of seeding, surface functionalised HPCs showed a significant increase in morphological markers of adhesion *in vitro*, with preferential increases in adhesion to certain ECM proteins. Furthermore, using a novel, gravity-driven microfluidic device, we demonstrate coated cell adhesion of HPCs to liver microtissues under flow. Altogether, these results demonstrate this technique as a viable method for increasing the adhesion of regenerative cells. The translatable nature of this technique to other cell candidates may have significant implications for the improvement of other cell-based therapies.

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RECAPITULATING THE LIVER TUMOUR ENDOTHELIUM *IN VITRO*: A KEY TOOL FOR NOVEL DRUG DELIVERY STUDIES

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Abstract theme: CDT Session

Introduction

Primary liver cancer, also known as hepatocellular carcinoma (HCC), represents a significant and increasing healthcare burden globally and effective therapeutic options are currently limited. Treatment failure is commonly attributed to the highly immunosuppressive tumour microenvironment and key contributors to this are the endothelial cells which line the tumour vasculature. These cells are critical in regulating the infiltration of immune cells from the circulation into the tumour. Studying these cells in more detail will be key to designing much needed novel therapeutics for the treatment of HCC tumours; however, isolating and culturing these cells *in vitro* is extremely challenging. Nevertheless, previous RNA-sequencing data from our group has demonstrated that HCC tumour endothelial cells possess a distinct phenotype, when compared to endothelia isolated from matched non-tumour tissues. In this project, we investigate the possibility of reprogramming primary human liver endothelial cells to a more tumour endothelial-like phenotype in order to aid the design of novel therapeutics for the treatment of HCC.

Methods

The expression of key markers enriched in HCC tumour endothelial cells were validated in HCC tumour tissues *ex vivo* via immunohistochemistry. Primary human liver endothelial cells were isolated from explanted diseased liver and rejected donor liver tissues. Briefly, this was achieved through mechanical and enzymatic digestion, density-gradient centrifugation and positive selection for $CD31^+$ endothelial cells via immunomagnetic beads. Isolated liver endothelial cells were then cultured *in vitro* and induced to express a tumour endothelial-like phenotype by treatment with a high dose (100 ng/ml) of vascular endothelial growth factor (VEGF) for 24 hours. HCC tumour endothelial markers were confirmed at a transcript level via PCR, and protein level via immunohistochemistry and western blot analyses.

Results

Here, we characterise tumour endothelial cells in hepatocellular carcinoma (HCC) tumour tissues *ex vivo*, by immunohistochemical staining of a panel of key markers, such as CD31, CD34, PLVAP, CD73 and stabilin-1. Utilising a high dose of VEGF (100 ng/ml) for 24 hours, we show the induction of mRNA expression of a number of tumour endothelial markers, such as *CD31*, *CD34*, *NT5E* and *PLVAP* in primary human liver endothelial cells *in vitro*. In addition, we use immunofluorescent staining and western blotting to confirm protein expression of the same markers.

Conclusion

Using treatment with a high dose of VEGF, we have managed to induce a liver tumour endothelial-like phenotype in primary human liver endothelial cells. We confirm this via qPCR analysis, immunofluorescent staining and western blot analyses. Future studies will aim to utilise this induced tumour endothelium phenotype to aid in the design of novel therapeutics for the treatment of HCC. Specifically, we will study the migration of cargo-loaded monocytes across the induced tumour endothelium under physiological flow conditions *in vitro*, with the intention of producing a novel drug delivery system to HCC tumours.

WEARABLE SENSORS FOR MEDICAL DIAGNOSTICS AND MONITORING HEALTH AND WELLBEING

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Abstract theme: CDT Session

Introduction

There is a growing need for systems that help monitor patients at home in a robust and continuous fashion. Tracking levels of biomarkers that are indicative of specific diseases or health conditions could have significant socioeconomic impact on healthcare systems globally, allowing for early diagnostics and personalised treatment. Wearable devices can allow for painless and comfortable real-time monitoring of a patient's condition, using sweat (1). Lactic acid is a molecule of interest in both the sports and healthcare fields since it can be an indicator of an athlete's physical status and of important medical conditions (2), including cardiac arrest and sepsis (3). In this project, we focus on the development of novel wearable devices for the non-invasive and continuous monitoring of lactic acid in sweat. Key challenges include improving the length of time in which these devices can be continuously used, from hours to days, as well as creating microfluidic channels that allow for sweat to be carried into the sensor, and the compatibility of different biomarker detection systems in one sensor.

Materials and methods

We present initial results on the benchmarking colorimetric assay used to calibrate our wearable designs. The assay uses an enzymatic reaction catalised by Lactate Oxidase (LOx) which converts lactic acid into pyruvate and H_2O_2. In a second reaction catalised by a peroxidase from horseradish, the chromogenic agent 2,2′-azino-di-[3-ethylbenzthiazoine-sulfonate] (ABTS) is oxidized by the produced H_2O_2. The oxidised ABTS changes colour and can be measured in a spectrophotometer at 420nm, and this oxidation is proportional to the initial lactic acid concentration (4). This colorimetric assay will be used to make a lactic acid standard curve, optimise the LOx concentration for our sensor and optimise the velocity of our reaction. These experiments will be repeated in an electrochemical system after binding LOx to screen printed electrodes, provided by our industry partners Zimmer & Peacock. A potentiostat will be used to analyse results.

Results and conclusions

Initial results of a lactic acid standard curve highlight the importance of optimising the ratio of the enzyme, lactic acid and ABTS. A Michaelis-Menten curve is produced, enabling us to fully characterise the enzyme system, to be ported onto the electrochemical wearable platform.

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A SYNTHETIC BONE MARROW NICHE MODEL FOR TESTING A NOVEL LEUKAEMIA TREATMENT

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Introduction: Haematopoietic stem cells (HSCs) are responsible for the process of haematopoiesis, the continuous production of blood and immune cells [1]. HSCs natively reside within the bone marrow (BM) niche, which maintains the stem cell pool and haematopoietic activity with a variety of stimuli [2]. When haematopoiesis goes awry it can result in diseases such as acute myeloid leukaemia (AML), which was responsible for ~93,000 deaths globally in 2019 [3]. Novel therapies have the potential to significantly improve AML patients' outlook [4]. These include chimeric antigen receptor T-cell (CART) therapies [5], one of which we are developing. However, the efficacy of novel therapies is difficult to test prior to and in some cases during *in vivo* trials [6]. To address this, we have developed a model BM niche that can provide insight into how well a therapy will work in a clinical setting.

Materials and methods: Our model uses mesenchymal stromal cells (MSCs), which coexist alongside the HSCs in the native BM niche [7] as a feeder layer. The MSCs are cultured on a surface coated with poly(ethyl acrylate), a monomer that causes fibronectin, which the surface will also be coated with, to assemble in an open conformation [8]. This allows the fibronectin molecules' integrin binding and growth factor binding domains, the latter of which is loaded with the osteogenic growth factor bone morphogenic protein 2, to synergistically signal the MSCs cultured on top of them [9]. A synthetic, peptide-based hydrogel [10] is also layered on top of the MSCs. HSCs are then cultured on top of the gel. We are also developing a CART therapy which specifically targets and eliminates AML cells. Established CART approaches to AML are hampered by the lack of differentiable surface markers that can be used to target AML cells without causing harm to a patient's healthy hematopoietic cells [11]. To overcome this, we have developed a method of ablating the non-functional myeloid cell surface marker CD33 [5] from HSCs. This marker is highly expressed on AML cells in most patients, making it ideal for CART targeting [12]. This system produces HSCs that are not targeted by CD33-redirected CART cells and could be implanted alongside CART cells to replace ablated myeloid cells.

Results and discussion: Our BM niche system mimics the biochemical and mechanical properties of the BM niche, encouraging MSCs to adopt a niche-like phenotype, as confirmed by analysis of key markers' expression. This system is also capable of maintaining HSCs in vivo to a similar standard as previously validated systems. We have also successfully knocked out CD33 in model cells and HSCs, making them resistant to CD33-redirected CART cells. To marry these two aspects of the project, the effectiveness of the aforementioned CART therapy within the *in vivo* BM niche was modeled using the synthetic BM niche developed.

Conclusion: To summarise, we have developed a system capable of acting as a platform for in vivo testing of therapies for BM-associated diseases, such as a CART system that targets CD33-presenting AML cells while sparing CRISPR edited HSCs and their progeny, which don't express CD33. The model BM system could be used to validate a litany of therapies prior to in vivo testing, while the therapy we are developing promises to improve AML patients' prognoses significantly.

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POLYSACCHARIDE-BASED HYDROGELS AS 3D SYSTEMS FOR MODELLING OF CHRONIC WOUNDS

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Abstract theme: Bioengineered Models / CDT Session

Introduction Chronic wounds pose a significant global challenge, with approximately 40-60% failing to heal within 3 months, necessitating clinical therapies like collagen-based dressings to manage excessive inflammation. Presently, the development of such treatments relies on 2D/3D organotypic models, which fall short in replicating the intricate structural and functional aspects of natural skin, impacting research quality and clinical outcomes. There's a pressing industry need for a model that reduces reliance on animal testing and accurately represents human skin physiology. Tissue engineering offers the possibility of combining cells with biomaterials and other biomolecules to create 3D models that better represent the extracellular matrix environment of native tissues. The bioprinting process helps assemble the materials and cells into 3D constructs with biomimetic structural and functional organization. Collagen and other materials, such as Hyaluronic acid, have previously been utilised in wound modeling. However, they present challenges such as high costs, replication difficulty, and unreliability. Furthermore, the 3D printing of collagen faces numerous obstacles, including issues with bioink control due to the process of thermal crosslinking and limited mechanical strength for creating durable structures. Dextran, a polysaccharide comprising of glucose molecules, has emerged as a promising polymeric ink in 3D bioprinting due to its favorable properties, including biocompatibility, viscosity, rheology, gelation, chemical modifiability, and biodegradability. Although hydrogels based on dextran have been employed in developing wound dressings, they have not been extensively utilised in skin modeling. Combining dextran-based hydrogels with a 3D bioprinting process could potentially yield more robust, reproducible, tunable, affordable and scalable models for skin tissue engineering

Materials & Methods In this study, we explore the utilisation of semi-synthetic dextran-based hydrogels as polymeric inks for 3D bioprinting of full-thickness skin models. Our methodology involves modifying Dextran with Glycidyl- Methacrylate to produce DexGMA, followed by characterisation using NMR and FTIR. We examine the rheological properties of each hydrogel and conduct photo-rheology to assess the curing kinetics of the photocrosslinkable DexGMA. Additionally, we carry out cell culture investigations to evaluate the biocompatibility of DexGMA with the chosen cell types.

Results & Discussion NMR and FTIR analyses confirm the successful in-house synthesis and modification of Dextran with GMA to obtain DexGMA. Mechanical testing and rheological assessments demonstrate the superior rapid crosslinking abilities of DexGMA hydrogels compared to collagen-based alternatives. Initial biocompatibility studies suggest that DexGMA is compatible with the selected cell types for our research. Efforts are presently ongoing to construct a two-layered, bioprinted skin model using DexGMA and to refine the composition of the bioink.

Conclusion Our findings suggest that DexGMA-based hydrogels offer several advantages over collagen-based counterparts when crafting and fabricating skin models, owing to their biocompatibility, adjustable mechanical characteristics, and enhanced printability. DexGMA-based hydrogels hold potential for meeting industry demands, enabling high throughput and reliable production. Our next step involves exploring the integration of various biological ECM components into our DexGMA hydrogel and evaluating the suitability of these semi- synthetic materials for printing a full-thickness chronic wound model that aligns with prevailing industry standards.

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ENGINEERING VISCOELASTIC HYDROGELS FOR MIMICKING THE TUMOUR MICROENVIRONMENT AND INVESTIGATING BREAST CANCER CELL MECHANOSENSING

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Abstract theme: Mechanobiology / Biomaterials / CDT Session

Abstract:

Introduction: Each year, approximately 15% of people worldwide, mostly women, are diagnosed with Breast Cancer (BC) which is the uncontrolled proliferation of cells leading to the formation of a tumour[1]. Several studies have shown that alterations in the surrounding area of cells, known as the extracellular matrix (ECM), contribute to the progression and metastasis of breast tumours. The breast cancer ECM undergoes cell- induced remodelling, including alterations in the expression of proteins such as fibronectin and collagen, as well as mechanical stiffening compared to the healthy stromal ECM[2].

Cancer research is proven to be complex and challenging for scientists and new in-vitro assays must be used to avoid animal studies. In recent years, 2D and 3D biomaterials, known as hydrogels, were developed to facilitate cells growth, and recapitulate the environmental ECM of cancer cells. However, although the role of ECM stiffness / elasticity is now well reported, the impact of ECM viscoelasticity on cancer cell behaviour is limited and still elusive. The aim of this research is to design and fabricate viscoelastic hydrogels with tuneable properties to (i) mimic the viscoelastic properties of the breast cancer microenvironment and (ii) understand how different cancer cell types sense both the elastic and viscoelastic properties of their environment. Methodology: 2D polyacrylamide hydrogels with different elastic and viscoelastic properties [soft (0.3kPa) Elastic/Viscoelastic & stiff (3kPa) Elastic/Viscoelastic] were fabricated and used as substrates for three breast cancer cell lines to adhere and grow[3]. Cell and nuclear morphology, focal adhesions and YAP expression were investigated and will be discussed.

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DEVELOPING PRINTABLE HYALURONIC ACID-HEPARIN GELS FOR SUSTAINED SUPPORT OF NEURAL CULTURES AUTHORS

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Introduction With our global population aging, neurodegenerative disease poses an increasingly greater risk and therefore *in vitro* brain modelling research is becoming increasingly crucial: CNS drug trials are failing; Animal models lack translatability; and Current models lack maturity and complexity. The brain microenvironment is vital for sustaining healthy neuronal function and is primarily controlled by the distribution of factors in the encompassing extracellular matrix. Matrix, architectural and molecular cues during corticogenesis also facilitate the complex layering and maturation of the brain in utero – as such, engineered incorporation of a select few could yield the translatable brain models needed.

Materials and Methods Using iPSC (Line 5) derived neural precursor cells, gel component optimisation will be achieved by MTT viability assays, quantification of neurite outgrowth and morphological analysis to establish working concentrations of heparin – a key gel component – molecular signals including neurodevelopmental cues and growth factors including BDNF and VEGF. Initial work in 2D requires NPCs grown on tissue culture plates, while 3D work incorporates the use of NPC spheroids created in Aggrewell plates (Stem Cell Technologies, Canada) and comparative commercial gels including HyStem and HyStem-Hep (Advanced Biomatrix, CA, USA). In developing our own hydrogel, various functionalised versions of the key polymers (hyaluronic acid and heparin; Creative PEGWorks, NC, USA) have been purchased alongside versions of the synthetic polymer PEG (Creative PEGWorks, NC, USA) and combinations will be synthesised into gels and analysed for rheological suitability (amplitude and frequency sweep testing), viability (Live/Dead staining), printability (resolution, set time), and molecular signalling availability (spatial analysis with fluorescent His tag labelling). Secondary photocrosslinking of the structures also requires MTT viability assays, looking at crosslinker concentration, UV intensity and the combination of those conditions.

Results and Discussion Findings so far indicate that some molecular cues analysed have a significant effect on neurite outgrowth of plated NPC spheroids, albeit without directionality. Moving into 3D, the HA-Heparin gel will be important for the continual exposure of these cues given the heparin binding region on the motif, allowing incorporation into the gel. Viability ranges for heparin concentration have also been established at maximally 2.5mg/ml. Having identified physical crosslinking as the primary crosslinking method with interaction between the functional groups selected, comparing different molecular weights, degrees of substitution and inclusion of synthetic polymers are expected to further influence the main optimisation points for the gel: printability, rheology, viability and molecular signalling availability[1]. Following printing, a secondary photocrosslinking stage to stabilise the gel for long term culture has been considered: viability assays with SH-SY5Ys to optimise crosslinker concentration, UV intensity and the combined conditions has shown that though UV and crosslinker exposure independently showed no effect on viability – it did significantly reduce viability when combining the two at a higher UV intensity and photocrosslinker (LAP) concentration (1.5i UV (0.45 Amps) at 0.1% crosslinker concentration).

Conclusions Though work is ongoing, initial optimisation of gel components (heparin concentration, crosslinker concentration, UV intensity) has set parameters within which viability of the neural cultures should be unaffected. Identifying the necessary molecular cues for incorporation into the gels have initially flagged key neurodevelopmental signals as important (though experimental repeats are necessary) and moving forward, growth factor selection as well as the release profile and distribution of molecular cues will crucially inform further optimisation. Each addition hopes to facilitate the sustained culture of functional neural co-cultures and enhance features of corticogenesis (particularly cortical layering) to improve the maturation of the cultures.

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Growing biohybrid scaffolds in the lab: Controlling the culture environment to create biohybrid scaffolds with pre-determined compositions and functionalities.

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Introduction Cardiovascular disease is the major cause of death globally, with over 17 million deaths worldwide. [1] Currently surgical procedures like coronary bypass surgery and percutaneous coronary interventions are the gold standard. However, these methods suffer from donor site morbidity and poor long term success. [2] Synthetic grafts are a possible alternative which whilst successful at large diameters, this solution has poor patency for diameters less than 6 mm and a lack of living cells limits the facilitation of new tissue growth, so tissue engineered vascular grafts (TEVG) are aimed to fill this demand. [3]

This project aims to optimise the previously developed method of poly(glycerol sebacate) methacrylate (PGS-M) high internal phase emulsions (HIPE) as a basis for the formation of biohybrid scaffolds for vascular replacement. The addition of a methacrylate group to the elastomeric PGS allows for UV crosslinking, which combined with emulsion templating and injection moulding allows for the manufacturingofporous,interconnectingscaffoldswithcomplexarchitectures.[4,5] Byvaryingconditions and reagents, high control over the synthesis will be obtained and will allow for tuneable, patient specific, off-the-shelf grafts.

Materials and Methods PGS and subsequently PGS-M have been synthesised in house via the two-step condensation reaction of equimolar amounts of sebacic acid and glycerol, followed by methacrylation to form PGS-M using methacrylic anhydride. Prior to UV curing, polyHIPEs of PGS-M were produced to create highly interconnected, porous scaffolds. The stirring speed, temperature and surfactant were varied to create scaffolds of differing morphology and architecture. The scaffolds are then cultured with human fibroblasts to generate a biohybrid scaffold containing both synthetic and cell derived components. The cellular response of BJ-5ta human fibroblasts was investigated to determine the optimum scaffold for the cell proliferation, ECM production and composition.

Results and Discussion PGS-M was synthesised through conventional synthesis methods and numerous polyHIPEs of this were produced by varying the parameters of the emulsion created scaffolds of different porosities and interconnectivities. After visualisation through scanning electron microscopy, the effect of each parameter has been determined. The culture of a human fibroblast cell line onto the scaffold has proved the ability of these scaffolds to support cell proliferation and ECM production. Further work will include an investigation into the effect of the porosity on volume and composition of the different ECM components.

Conclusions PolyHIPE scaffolds of various morphologies have been synthesised and characterised to begin investigation into the effect of cell environments on the production of ECM components. The impact of each component has been determined and further investigation will allow understanding of how the scaffold structure and culture environment influences ECM production.

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Abstract theme: CDT Session

EDIBLE BLENDS OF SILK FIBROIN FOR BIOFABRICATION OF ANIMAL TISSUE

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Introduction: Food security is amongst the most prominent humanitarian crises the world has faced, with the UN reporting approximately 828 million people suffering with hunger in 2021(1). Traditional food production practices are major contributors to greenhouse gas emissions, deforestation and loss of biodiversity and with the predicted increase in global population to almost 10 billion by 2050, the need to find sustainable food production alternatives is imminent (2). Cultivated (or lab-grown) meat promises to address the ethical, safety and potentially environmental concerns associated with the meat industry (3). 3D printing can be viewed as a possible manufacturing method that is able to reliably produce constructs that successfully mimic *in vitro* native tissue and the organoleptic properties of meat. As a cheap, abundant, edible, cell safe, non-mammalian derived protein, silk fibroin (SF) is a good candidate for cultivated meat scaffolds and has already been used in tissue engineering applications (4). Although SF is a well suited material for 3D printing, it suffers from some drawbacks, *e.g.* it is not viscous enough to be printed alone, and its high stiffness negatively affects cell attachment, proliferation and differentiation. In this work we are exploring ways to facilitate the 3D printing process of SF, so that the final scaffold is softer and therefore preferable for cell growth, whilst helping ensure the likely safety and compliance of these constructs as novel foods.

Materials and Methods: Different Various methods were tested to characterise SF gelation and viable print methodology, including sonication, ethanol (EtOH) print bath, and the use of cross-linkers. Concentrations of SF were tested at a range between 5 and 8% w/w. SF was also blended with other materials (such as zein) to functionalise it and tailor its mechanical properties. The supports were characterised by Fourier-transform infrared (FT-IR) spectroscopy to identify changes in the protein secondary structure and also tested for cell viability using bovine adipose derived mesenchymal stem cells. The 3D printing was done on a GRAPE S1 4D bioprinter.

Results and discussion: Sonication (both at room temperature and at 37 °C) appeared to have no effect on the SF solution characteristics, and it remained liquid throughout with no signs of gelation. Furthermore chemical characterisation did not reveal β-sheet formation which is contrary to what has been reported in the literature (4). EtOH on the other hand resulted in rapid gelation, as a consequence of the hydrophobic dehydration due to the interaction of the polar solvent and the protein chains (5). Optimal parameters for single layer prints were found but showed limited repeatability. Furthermore, the consistent gelation did not improve the printability of SF, which can be attributed to the low viscosity of the solution. Natural cross-linkers were also tried, as well as blends with polysaccharides at different print speeds and pressures. The main polysaccharide candidates were zein, arabinoxylan and dextrin and were chosen because they showed promising results in the literature for cell adhesion and proliferation (4). A shear-thinning material, Pluronic F- 127, was employed to normalise all results for quantitative comparison. In parallel, the viability of bovine mesenchymal stem cells whilst in contact with these materials was also demonstrated, to ensure their biocompatibility prior to further development.

Conclusions: Silk fibroin is a promising natural, abundant and edible material for cultivated meat applications, but poses certain challenges when it is used for 3D printing. Initial attempts for gelation (and therefore ease of printing) gave mixed results and highlighted the need for further optimisation. Blending SF with natural polysaccharides can improve surface functionalisation and provide the necessary environment for the viability of bovine cells.

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LINKING CELL GLYCOME CHANGES TO RHEUMATOID ARTHRITIS PHENOTYPES VIA RAMAN SPECTROSCOPY

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Introduction:
Rheumatoid arthritis (RA) is a degenerative autoimmune disease, affecting approximately 80 million people worldwide. The main target is the joint, inducing pain and stiffness, but disease present highly heterogenous pathophysiology, extraarticular symptoms and response to drugs. Around 5-20% of patients are deemed difficult to treat, due to unresponsiveness to medication and progression of symptoms [1]. These differing disease phenotypes are likely to be the cause for different degree of response to treatment.

Healthy synovial fibroblasts are responsible for maintaining ECM of synovial fluid in the joint. However, in RA, synovial fibroblasts become activated and undergo epigenetic changes to perpetuate local inflammation by releasing inflammatory mediators and recruiting immune cells to the joint. The molecular pathways controlling fibroblast activation are not fully understood. Previous work in the lab showed that reduction of sialic acid (SA) upon TNF exposure is sufficient to transform healthy fibroblasts into activated cells. This may be related to the biological role of surface SA-containing glycoconjugates in cell-cell interaction and signalling [2].

This study will observe the changes in synovial fibroblast glycome, focusing on levels of SA, and link it to varying RA phenotypes. For this, Raman spectroscopy will be applied to investigate whether RAMAN signatures of activated synovial fibroblasts correlate with cell activity and inflammatory stages. As Raman is fast, label-free and non-destructive to samples, it could provide a novel tool for differentiating between different RA phenotypes, based on the amount of SA present on cells.

Materials and Methods:
Human fibroblasts from synovial and non-synovial tissues were used as a model to investigate the presence of sialic acid; immunofluorescence (IF) and in-cell western (ICW) assay were performed. Biotinylated *Sambucus Nigra* lectin was selected as a primary detection reagent due to its high affinity to sialic acid. It was added to the samples alongside DAPI and Streptavidin Alexa Fluor 555 for IF, and CellTag 520 and IRDye 800CW Streptavidin stains for ICW assay. For IF, all cells were permeabilised with 0.05% PBS Triton.
Furthermore, literature was reviewed for Raman applications in sialic acid detection and inflammatory conditions. To provide initial proof of concept, the proteins fetuin and asialofetuin (latter without sialic acid) were analysed with Horiba Raman spectrometer, using 532nm laser wavelength.

Results and Discussion:
Our results confirmed the presence of sialic acid on fibroblasts, exhibiting fluorescence on cell membrane and Golgi apparatus, in line with the presence of sialic acid on the outside and inside of the cells. Future work will include experiments with lectins showing complementary affinities, in resting and in vitro activated fibroblasts. Additionally, fibroblasts from more cell lines will be tested in aforementioned conditions.
Raman spectroscopy shows potential to improve diagnostic process of RA, as it offers initial signs of differentiating between complex molecules. The obtained spectra of the compounds were very similar with a difference of an intense peak observed around 1000cm^{-1} in fetuin, which could be indicative of chemical bonds present in sialic acid. Further testing by analysing pure sialic acid would be able to confirm this.

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ENHANCED MSC GROWTH USING POLYMERS THAT ORGANISE GROWTH FACTORS

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Introduction: In recent decades, mesenchymal stromal cells (MSCs) have garnered the attention of the scientific community on the premise that a range of therapies can be provided by tapping the immunomodulatory and differentiation capacities of these cells[1]. MSCs are non-hematopoietic stromal cells, meaning they are not involved in the production or development of blood cells within the bone marrow[2]. A major problem is that as MSCs are expanded ex-vivo, they lose their differentiation potential and stop growing[3]. To unlock the regenerative potential of MSCs and translate this potential clinically into various therapeutic approaches, researchers must overcome these obstacles associated with the longevity and, ultimately, engraftment capabilities of these cells. Proteins found within the extracellular matrix (ECM) contain integrin binding regions that promote cell adhesion and heparin-II binding sites that can strongly interact with a multitude of growth factors[4]. Indeed, the ECM mesh has been shown to gradually release the desired growth factors, allowing for MSCs to differentiate into desired cell lineages[5]. Researchers have therefore started to become interested in finding a way to control the release of growth factors when using biomaterials with the aim of controlling the growth of MSCs to allow delivery of therapeutic numbers – before control of differentiation. Here, we employ the polymer poly (ethyl acrylate) (PEA) as it has been shown to allow molecules of ECM proteins like laminin (LM) to self-assemble on the surface and form nanonetworks, in such a way that the growth factor binding domain and the integrin binding domain within the protein was readily exposed to growth factors[6].

Materials and Methods: MSCs were cultured for 21 days in 24-well plates coated with PEA and treated with LM at a concentration of 20µg/ml. Immunofluorescence, RNA analysis and protein assays were employed to assess the expression levels of markers associated with MSC growth and proliferation. The alamarBlue proliferation assay was used to quantify MSC growth over the experimental period. All experiments were conducted using a control surface of tissue culture plastic for an accurate comparison on the synergistic effects that PEA and LM pose to MSC growth.

Results and Discussion: Data from the alamarBlue proliferation assay indicates that there was a significantly higher percentage reduction in samples where MSCs were grown on LM coated PEA compared to tissue culture plastics, adding to the notion that LM could pose as a significant role in maintaining MSC viability. This is an important finding because as cells proliferate, they have an increased metabolic demand that experimentally translates to a higher percentage reduction in alamarBlue. Additionally, preliminary data using qRT-PCR has shown a decreasing trend in the fold change in growth-related markers in MSCs grown on PEA surfaces coated with LM. Further studies at RNA and protein level are necessary to elucidate the exact nature of the aforementioned mechanism.

Conclusions: The data obtained thus far raises an exciting question pertaining to whether structured and controlled release of growth factors can be achieved using LM coated PEA, that could potentially be used to regulate MSC differentiation, with a view at subsequent targeted therapeutic approaches.

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MODELLING MECHANICAL PROPERTIES OF HYDROGELS FOR PRECISION IN-VITRO SCAFFOLD DEVELOPMENT

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Introduction The extracellular matrix (ECM) is a three-dimensional structure that provides mechanical and biochemical support, directing the phenotypes of surrounding cells, and is highly involved in biological mechanisms like disease development and progression.

Viscoelasticity has been widely identified as a mechanical property of interest involved in cell-extracellular matrix interactions. This interaction is frequently deregulated in the tumour microenvironment during cancer progression. Developing in-vitro models that can independently tune viscoelasticity, within a range mapped to that of patient tissues, is key to further investigating their effect in normal and diseased states. To directly compare properties of human tissue samples and hydrogels, a method of analysing the mechanical properties with respect to viscoelasticity is required.

Materials and Methods Composite hydrogels of pristine alginate, OA, gelatin, and PEG, with calcium chloride as a crosslinker, successfully create tuneable hydrogels capable of mimicking the mechanical properties of soft human tissues and tumours. By varying the degree of oxidation of OA, molecular weight of PEG, and concentration of ionic crosslinkers, a range of target mechanical properties can be mapped independently of the biochemical properties. This enables facile testing of the effect of viscoelasticity on the cell-substrate interaction in-vitro.

These materials have been investigated through rheology and compression. To directly compare viscoelasticity between samples, a standardised method of testing and analysis has been developed. A computer model uses raw data from mechanical tests (from hydrogels and human tissue samples) and calculates values for the elastic, viscous, and viscoelastic elements of the sample. As cell-substrate interactions take place over short time periods (~1s), the amount of energy released immediately after removal of the applied stress is used to quantify viscoelasticity.

In this study, the different stages of viscoelastic behaviour during a creep-relaxation cycle were identified and used to produce a mathematical model able to identify viscoelastic parameters. The model combines the Maxwell and Kelvin mathematical models: a spring, dashpot, or a combination of both to map the fully elastic, fully viscous, and time-dependant responses respectively.

Results For the hydrogel samples, between 0.01% and 0.5% strain energy is released within the first 0.5s in either shear or compression, which is in line with the values from human tissues. The lower the energy released, the more viscous the material. Increasing the molecular weight of PEG increases viscosity, while increasing calcium crosslinking increases elasticity. The order of magnitude difference between the values demonstrates that the mechanical environment experienced by the cells varies, impacting the mechanical cues and therefore the cellular response. The model also calculates the relaxation time, an important parameter in understanding the viscoelastic effect during cell-substrate interactions. A small library of these alginate-based hydrogels is being produced, where viscoelasticity is varied independently of the other mechanical properties, and the effect of different relaxation rates on the cell- substrate interaction can be assessed.

Discussion Composite alginate hydrogels are highly versatile substrates for in-vitro models capable of mimicking a wide range of tissue-specific microenvironments and enabling enhanced understanding of the role viscoelasticity plays in cellular behaviour and disease progression. By varying the composition of the hydrogel, the viscoelasticity of the resulting substrate is altered within a platform with consistent chemical and biological properties. The next step is to investigate the effect of this change in viscoelasticity on cell behaviour, such as proliferation, migration, and adhesion.

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ORGAN-ON-CHIP: ANIMAL-FREE METHODS FOR DRUG SAFETY TESTING

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Introduction

One of the most pressing issues in drug development at present is the rate of failure of new drugs – presenting a massive financial loss and wasted resources. Around 90% of new drugs fail at some stage of development, with around 30% of these failures stemming from issues with toxicity (1). This has called into question current methods of drug testing and taken alongside growing concerns about the translatability of conventional animal models to humans (2), there is an increasing interest in developing novel animal-free methodologies – which may lead to more physiologically-relevant, safe drugs passing through the drug development pipeline and reaching the market. One such new method is "organ-on-a-chip" – which combines microfluidics and cell culture to mimic the *in vivo* environment more closely than traditional *in vitro* methods. Organ-on-a-chip technology has a broad spectrum of applications, but a particularly noteworthy application has been in the prediction of drug-induced liver injury (DILI) – where this model has proved a better indicator than conventional in vivo animal models (3). This project aims to develop organ-on-a-chip based models of the liver using 3D spheroids, and – through advanced spectroscopic methods such as Raman spectroscopy – investigate the pharmacokinetics and metabolism of compounds within these spheroids. This project will utilise animal-free culture methods and cell lines, working within the framework and guidelines established by our charity partner Animal Free Research UK (AFRUK).

Materials and Methods

This project will work with microfluidics-based systems ("organ-on-a-chip"), where nanofabricated masks will be designed to allow for cell culture and the application of fluid flow. Once constructed, these nanofabricated masks can then be used to make multiple chips made of polydimethylsiloxane (PDMS). These PDMS chips can be hooked up to pumping and draining systems through their inlets and outlets, allowing the rate of flow through the chip to be adjusted as desired. The clear surface of the PDMS chips allows for these to be easily imaged, whether through microscopy or Raman spectroscopy. Cells will be seeded and cultured within these chips and measured against various parameters to assess viability and function.

Results and Conclusions

Experiments are still in progress – however results will investigate the viability and functionality of cells cultured for various timepoints within these microfluidic devices with and without compounds of interest. Results including figures and subsequent conclusions will be included in the final poster.

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FROM BEE'S KNEES TO BIOTECHNOLOGY

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Introduction: Tissue engineering has been extensively used in biomedical engineering applications. Hydrogels are polymeric gels commonly used in cell culture and tissue engineering, which exist in both solid and liquid phases.[1] These materials can be natural, such as collagen, synthetics, like Polyethylene glycol (PEG), or a combination of both.[2] Hydrogels represent attractive scaffold materials since they have similar topologies to the extracellular matrix of many tissue types and their properties can determine cell fate.[1,2] However, nowadays most approaches use hydrogels derived from animal products.[3] This project focuses on an animal-free approach where a commonly used polymer, PEG, and a synthesised resilin-like polypeptide are combined to fabricate hydrogels for cell culture and bioprinting. Resilin is an elastomeric protein which belongs to the same family as elastin, with remarkable mechanical properties and efficient energy storage.[4,5] This protein is found in insect cuticles and is literally the bee's knees. Resilin-based proteins can be tailored for specific applications. Specifically in tissue engineering, proteins with resilin-based sequences and bioactive domains can result in hydrogels with the superior mechanical and physical properties of resilin capable of controlling cell response.[5] PEG is a synthetic, biodegradable, and water-soluble polyether widely used in biomaterials due to its biocompatibility and extreme hydrophilicity.[6,7]

Materials and Methods: UniprotTM was used to find repeat motifs of resilin which were then input in WebLogo to create the amino acid sequence PSDSYGAPGGGN as a model peptide. Then, Solid Phase Peptide Synthesis was performed using the CEM Microwave Peptide Synthesiser. The sequence of the model peptide, YGAP1, created was Ac-RPSDSYGAP-NH_2. High-Performance Liquid Chromatography (HPLC) and Liquid Chromatography Mass Spectrometry (LC-MS) tests were performed for quality control and identification, respectively. Following that, a portion of YGAP1 was used to make a peptide with the same sequence and alkyne side chain on the N-terminus by manually coupling Fmoc-propargyl glycine. After, a cleave reaction was performed to convert the amine end to an amide, followed by HLPC and LC-MS purification. Conditions were established to achieve triazole formation followed by HPLC purification to confirm its formation. Regarding the polymer project, experiments for a functionalised Polyethylene Glycol (PEG) synthesis were performed as well as monomer polymerisations. PEG2000 was used for a reaction with Bromoacetyl Bromide and the addition of Triethylamine to make up PEG-Br. Using Sodium azide, an attempt to convert Bromide to azide was performed. Proton Nuclear Magnetic Resonance (1H NMR) tests were performed, as well as Electrospray Ionisation Mass Spectrometry (ESI-MS). A different reaction in which methanesulfonyl chloride was used instead of Bromoacetyl Bromide was also performed. Reversible Addition Fragmentation Chain Transfer (RAFT) polymerisation was utilised for the conversion of Pentafluorophenylmethacrylate (PFMA) monomer to a polymer. Gel permeation chromatography (GPC) for PFMA polymerisation was then performed to identify the polydispersity to calculate a 10% displacement with Tyramine which was then performed. **Results and Discussion:** The peptides synthesised so far have been successful and the YGAP1 sequence presents as a promising starting material for producing a suitable resilin-like polypeptide. PFMA polymerisation was confirmed by 1H NMR tests. 1H NMR and ESI-MS tests of the PEG-methanesulfonyl chloride reaction product are required to identify the favourable functionalised PEG synthesis reaction.

Conclusions: Most hydrogels used in biomedical applications are fabricated using animal products. Resilin-based hydrogels are animal-free and offer great potential for cell culture and bioprinting. The approach described in this project involves the crosslinking of a PEG polymer with azide side chains, with a resilin-like polypeptide. Future work includes the synthesis of a peptide with alkyne ends on both termini for crosslinking with the PEG-polymer as well as the synthesis of a peptide with an azide end to test out crosslinking the peptides between them. 1H NMR will also be performed for PFMA-Tyramine. Tyramine is similar to Tyrosine, which will be involved in crosslinking with the resilin-like polypeptide. More experiments are required to determine the preferred chain length of the PFMA polymer, and the quantity of Tyramine to be displaced.

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ADVANCED VISCOELASTIC 3D BIOPRINTED ALGINATE/DNA SCAFFOLDS FOR STEM CELL ENGINEERING

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Abstract theme: Bioengineered Models / Mechanobiology / Enabling Technologies / Biomaterials / CDT Session

Introduction:

Extrusion 3D bioprinting is a method of scaffold fabrication that allows for predesigned scaffold structure with the combined ability to include multiple ink and cell types in one construct. This technology offers patient specific tissue models and enables the production of complex architectures, therefore enhancing biomimicry of tissue specific structure and function. The bioinks required for 3D bioprinting using extrusion-based methods must have certain properties such as having shear thinning behaviour, a temperature dependent viscosity and ability to form a gel after printing. Alginate is a natural polysaccharide that can be used in bioprinting and subsequently ionically crosslinked to form a gel, however pure alginate inks are not able to be printed due to their low viscosity at printing temperatures and their inability to maintain shape fidelity post printing. In this work we hypothesise a method of thickening unprintable alginate using DNA with consequential removal of this thickener post printing and crosslinking using DNase, leaving a pure self-supported tissue scaffold. By utilising the different molecular weights of alginate and DNA, a family of printable bioinks with different viscoelasticity's will be obtained to ultimately create bioinks that can support and drive stem cell differentiation, closely mimicking the mechanical properties of native tissue.

Materials and Methods:

DNA was isolated from *E. Coli* Nissle following the mini prep protocol. The extracted DNA was then reconstituted in illusion buffer to then be used to the stock solution, and was stored at 4° C. The alginate DNA gels were fabricated by gently mixing different ratios of alginate and DNA before inserting into a syringe. Rheological sweeps were used to assess the viscosity of the gel solutions with increasing shear rates up to 300m/s repeated for differing temperatures (37°C - 25°C).

Results and Discussion:

Alginate DNA bioinks were shown to have a shear thinning behaviour and a temperature dependent viscosity, offering initial signs of suitable use as bioinks in extrusion 3D bioprinting. The precipitation of DNA when mixed with the alginate results in an inhomogeneous gel solution, so the inclusion of restriction enzymes to reach the length of the DNA molecule will be investigated.

POLYHYDROXYALKANOATE (PHA) BASED 3D IN VITRO LUNG MODEL

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Abstract theme: Bioengineered Models

Introduction:

With the steady increase in incidences of lung diseases and the recent COVID-19 pandemic, a better understanding of the pathophysiology of lung illnesses is needed. The lung itself relies on a large surface area, extensive vasculature, and a complex cellular environment to facilitate gaseous exchange and protection from pathogens. This supported by an extracellular matrix (ECM) made from proteins that facilitate optimal spatial orientation and morphology of the native and immune cells whilst also providing structural integrity(1). The closest mimetics of this complex structure are animal models which are a depleting and expensive resource. Developing clinically relevant and reproducible models is now considered an important direction in understanding of lung diseases.

Polyhydroxyalkanoates (PHAs) are an FDA approved, sustainable family of polyesters; derived from bacterial fermentation under nutrient limiting conditions. PHAs are favoured for biomedical applications due their low immunogenicity, biocompatibility and tunability(2). Medium-chain PHAs (mcl-PHAs) have seen success in soft tissue applications from cardiac, wound healing alongside as cancer models(3). Inherent properties and recent applications show promise in replicating the mechanical and physical properties of lung ECM structures using PHAs for a subsequent disease model as shown below.

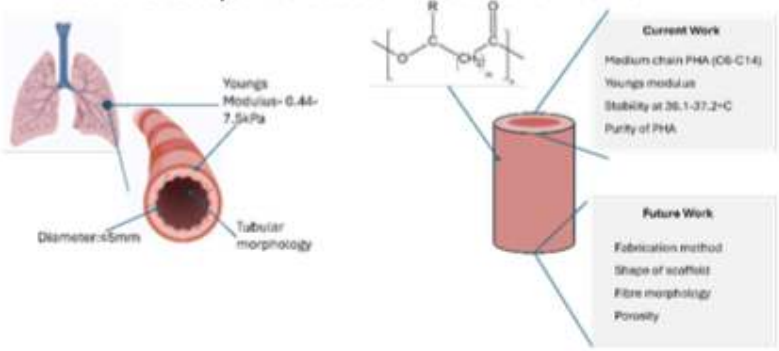

Figure 1 shows the mechanical properties of small airways in the lung and the considerations needed to be made when producing a PHA based lung model.

Materials and Methods

A selected *Pseudomonas* sp. was subjected to a batch fermentation using a stirred tank bioreactor. The inoculation was made using two-stage shaken flask fermentation; utilising nutrient broth as first stage and mineral salt for the second stage. The fermentation run for 48 hours with 20 g L^{-1} of glucose as a sole carbon source. The fermentation broth was centrifuged to harvest the cells in the form of pellet, and lyophilised. Dried biomass was subjected to the Soxhlet extraction method, and to obtain pure medical grade polymer. The dried solvent-cast polymer was subjected to several characterisation to determine its physical, thermal and chemical properties.

Results and Discussion

The polymer was characterised, and it exhibited an elastomeric property with 215 ± 52 % elongation at break with Young's modulus 15 ± 5 MPa from tensile testing analysis. Normal lung tissue varies between 0.44- 7.5 kPa(1) therefore modifications would need to be considered to adjust this product to fit this range. The PHA also had a low melting point, T_m of around 55 °C, making it processable with various fabrication methods; which can also alter T_m values. From GC-MS analysis, there were five different monomers detected, ranging from six to 14-carbon, making the polymer a mcl-PHA. The polymer obtain was pure, determined from ^{1}H NMR analysis.

Conclusions

Properties of mcl-PHA derived from *Pseudomonas* sp. are pure enough to be used as a scaffold
Youngs modulus falls slightly higher than the value for small airways so modifications needed
Next steps will involve testing fabrication techniques by producing a tubular scaffold

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EXPLOITING METABOLITE GPCR MECHANOTRANSDUCTION TO FIND NEW TREATMENTS FOR METABOLIC DISORDERS

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Introduction

Adipose tissue is known for its ability to uptake or release fat in the form of fatty acids and glycerol to be used in energy metabolism. However, energy metabolism is dysregulated in individuals that suffer from obesity and type 2 diabetes. Approximately 67% of adults in Scotland are overweight with 35% being obese[1]. As such, new treatment options are needed to combat this high level of obesity. Currently, high throughput assays used for drug discovery currently do not replicate disease states in patients. An area of interest is cellular mechanotransduction and its role in obesity is currently unknown. G protein-coupled receptors (GPCRs), a family of receptors that have been shown to be mechanosensitive. This project looks at how adipocyte mechanotransduction and interactions with their microenvironment dictates cellular signaling, specifically through metabolite GPCRs, GPCRs that bind to metabolites produced from energy metabolism.

Materials/Method

3T3-L1 cells, a mouse-derived fibroblast-like cell line, were used as a model to study adipogenesis. Atomic force microscopy (AFM) was used to study the topographical changes during differentiation over a 4-day period. AFM was also used during this 4-day period to measure the changes in stiffness of the cells during the initial stages of differentiation.

3T3-L1 cells were seeded onto with differing substrates to study changes in GPCR expression and adipogenesis to ensure function and gene expression were constant on different experimental substrates. Cells were seeded on either of the following: glass, plastic, glass and Type I collagen, and plastic and Type I collagen. RNA was isolated from 3T3-L1 cells before and after differentiation then quantitative PCR was used to measure the changes in RNA expression in FFA1, FFA4 and HCA2. between the substrates following differentiation. To investigate adipogenesis, Oil Red-O, a lipid stain, was used to measure the changes of lipid accumulation 14 days after differentiation, a standard used to measure adipocyte differentiation between the cells seeded on the different substrates of differing stiffness.

Results and Discussion

The initiation of differentiation showed topographical changes on the surface of 3T3-L1 cells. Although there are changes to the topography of the cell, they do not begin accumulating lipid at this stage of differentiation. The stiffness of these cells does not significantly change at these time points as they do not accumulate lipid. Quantitative PCR showed FFA1, FFA4 and HCA2 expression of the cells seeded on the varying substrates did not have any significant difference in the fold change of gene expression. There was no significant difference in adipogenesis across the varying substrates. This suggests the change in stiffness between glass and plastic does not affect adipogenesis or metabolite GPCR expression despite the addition of an extracellular matrix protein (Type I collagen).

Conclusion

Although the stiffness of 3T3-L1 cells are unchanged 4 days following differentiation, there are topographical changes of these cells. This suggests lipid accumulation must occur after 4 days of differentiation but there are changes to the morphology of the cells as they undergo adipogenesis. It should also be noted that GPCR expression and adipogenesis are unchanged when plated on substrates of high stiffness. This allows future work to be completed with the use of both plastic and glass as function and GPCR expression remain unchanged between them.

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INVESTIGATING THE ROLE OF THE BRAIN-MENINGES INTERFACE IN TRAUMATIC BRAIN INJURY

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Abstract theme: Bioengineered Models / CDT Session

Introduction:

Traumatic brain injury (TBI) is a serious health issue with approximately 50 million people suffering from a TBI each year. TBI has a complex pathophysiology with no effective therapeutics with significant long-term consequences including cognitive decline. The barriers of the brain have been increasingly recognised for their important roles in brain homeostasis and disease(1). These barriers also prevent therapeutics entering the brain which is a considerable issue. However, in comparison to barriers such as the blood-brain barrier, the brain-meninges interface is largely understudied. This interface is thought to be important in various disorders such as TBI where there are alterations in intracranial pressure which affect the tissue structures. Furthermore, recent evidence has shown an important immunological function of the meninges including interaction with astrocytes(2). This interaction may be key to improving the barrier functions of the primary human leptomeningeal cells, which have previously shown low TEER values(3), therefore better modelling the brain-meninges interface.

Therefore, the main aim of my project is to develop an *in vitro* model of this interface using primary human leptomeningeal cells and astrocytes. Due to the limited literature on the co-culturing of these cells together, an optimisation of cell culture conditions will be conducted.

Materials and methods:

Using direct co-culture, various medias will be tested to select optimal conditions for the leptomeningeal cells and astrocytes together and comparing to monoculture. I will be examining astrocyte medium, leptomeningeal cell medium, low glucose DMEM, along with testing different ratios of the mixed medias. Cell viability will be analysed using the MTT assay and AlamarBlue. The cell growth patterns will also be analysed using xCelligence, which is a real-time cell analysis system. Following this, immunofluorescent staining will be done to examine morphology and protein expression.

Results and discussion:

Preliminary data has shown that the different mediums have a considerable impact on cell growth in both monoculture and co-culture. Low-glucose DMEM was found to negatively impact cell growth shown by low cell index value (Fig 1.) and morphology of both cell types in contrast to the recommended astrocyte media. Continuous testing of media composition, seeding densities, substrates, and timings will be conducted. Selecting the correct medium is a challenge but important step in developing a mimetic *in vitro* model(4).

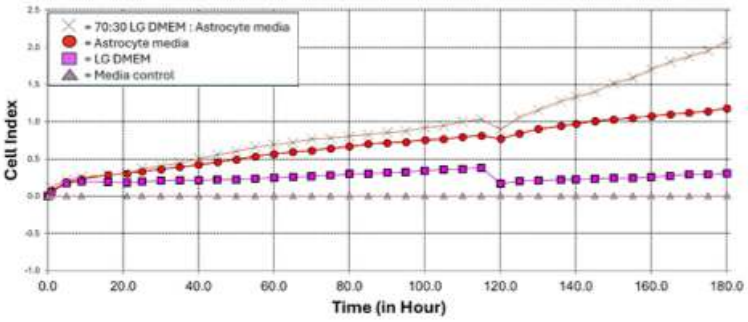

Figure 1: Preliminary data from xCelligence showing the growth patterns of primary human astrocytes over one week in different media. (LG DMEM = Low glucose Dulbecco's Modified Eagle Medium).

Conclusion:

Overall, the brain-meninges interface is an understudied barrier in the brain and this *in vitro* co-culture model may have important implications in drug screening applications and the understanding of both homeostatic and pathophysiological conditions of the brain. Future work will include using polydimethylsiloxane (PDMS) to mimic the mechanical and structural environment of both the brain (~3kPa) and the leptomeninges (~8MPa) and to determine the role of stiffness changes on these cells.

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THE ROLE OF MATRIX STIFFNESS IN PROTEIN FOLDING MACHINERIES

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Introduction: Adherent cells residing within the extracellular matrix respond to mechanical cues, regulating cell signalling and function. These mechanical cues are transduced through the cell's cytoskeleton and translated into biochemical signals via *mechanotransduction*[1]. While mechanotransduction has been linked to significant effects on gene expression and cellular behaviours, such as, migration, proliferation, and differentiation[2], its role in protein folding remains largely unexplored. In vivo, small chaperone molecules aid in the folding of proteins into their three-dimensional structures, impacting their functionality and determining their involvement in diverse biological processes and pathways. Any disruption in this process may result in different pathological conditions or physiological abnormalities[3]. Therefore, the aim of this study is to investigate the relationship between mechanotransduction and chaperones that governs protein folding capacity within the endoplasmic reticulum.

Materials and Methods: Polyacrylamide hydrogels with varying stiffness (300 Pa, 10 kPa, 30 kPa) were engineered to provide mechanical cues to immortalized mesenchymal stem cells (Y201)[3]. The hydrogels were functionalized with collagen and fibronectin, two key extracellular matrix protein facilitating cell adhesion. Over three distinct time points, cells were cultured and the protein levels of collagen type I and HSP47, a chaperone crucial for collagen folding and secretion, were evaluated using both western blot and immunofluorescence techniques.

Results and Discussion: The investigation involved the expression levels of collagen I and HSP47 across three stiffness conditions (300 Pa, 10 kPa, 30kPa) at multiple time points. Western blot analysis revealed distinct expression profiles of collagen I and HSP47 in cells cultured on hydrogels with varying stiffness. This response could be mediated by the molecular clutch model composed of integrin receptors, F-actin and adapter proteins, whereby cells can sense the mechanical rigidity of their environment and respond accordingly. Furthermore, differences in collagen I and HSP47 expression was observed between early (day 3) and later (day 7) time points, with higher expression observed at day 7 compared to day 3. These findings suggest dynamic cellular responses, possibly related to adaptation or remodeling processes[4]. Subsequent, immunofluorescence analysis is needed to further validate these findings, unveiling variations in both the secretion and distribution of collagen I in cells cultured on hydrogels of differing stiffness.

Conclusion: Our preliminary findings suggest a potential link between mechanotransduction and the expression of chaperones, such as HSP47, which could in turn influence the folding and loading of collagen into secretory vesicles for secretion. However, further experiments are required to conclusively determine whether the observed differences in expression levels of collagen I and HSP47 are indeed attributable to the varying stiffness of the hydrogels or potentially influenced by the specific ligands used as functionalizing agents for cell attachment. Future investigations will focus on elucidating the underlying mechanism by which mechanical cues modulate the expression of HSP47 and other chaperones, such as, BiP, Grp94, and TANGO1.

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PRECISION BIOFABRICATION FOR MENISCAL TISSUE ENGINEERING: LEVERAGING MEW AND MICROVALVE BIOPRINTING TO ENABLE TUNEABLE MECHANICAL AND CELLULAR GRADIENTS.

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Introduction: This project addresses the critical challenge of meniscal injuries affecting over 1.5 million individuals annually in the USA and Europe, often leading to early osteoarthritis. With existing surgical treatments showing limited success in tissue regeneration, the need for innovative approaches becomes imperative.

Materials and Methods: Our project centres on a sophisticated biofabrication pipeline, seamlessly integrating melt-electrowriting (MEW) and microvalve bioprinting techniques to craft meniscal tissue analogues containing cellular and mechanical gradients mirroring native tissue. Optimized MEW process parameters facilitated the deposition of layers of stiff polymeric microfibres, creating microchambers that reinforced a soft hydrogel matrix. The mechanical properties of this scaffold, pivotal for mimicking native tissue, underwent rigorous evaluation through compression tests. Microvalve jetting of alginate into MEW microchambers allowed precise droplet patterning, enabling the encapsulation of human MSCs with high viability.

Results and discussion: Our project achieved the production of aligned polymeric fibre walls emulating collagen bundles in native tissues, offering tuneable fibre diameters and microchamber sizes. Fine-tuning fibre diameter and spacing resulted in scaffolds approaching physiologically relevant stiffness, potentially allowing us to tailor the mechanosensory environment for encapsulated cells. Multi-nozzle microvalve jetting attained intricate patterns of bioink droplets within polymeric microchambers, showcasing high cell viability and enabling precise patterning of cellular gradients. After 28 days in culture, the reinforced scaffolds closely emulated the compressive stiffness of native cartilaginous tissue, featuring aligned collagen deposition along the polymeric fibres and glycosaminoglycan (GAG) deposition within the hydrogel matrix.

Conclusion: These hybrid constructs exhibited significant potential for engineering mechanically robust, cartilaginous tissue analogues, promising advancements in tissue regeneration and preclinical disease modelling.

TISSUE ENGINEERED BLOOD VESSELS

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Introduction Cardiovascular disease (CVD) is the most common cause of death worldwide, with incidence of the disease increasing annually.[1] CVD is often a result of atherosclerosis leading to vessels stenosis. The current gold- standard treatment is a coronary bypass surgery (CABG) where the occluded vessel is bypassed using an autologous (most commonly saphenous vein, SV) or synthetic vessel grafting to revascularize the region.[2] However, use of SV is associated with donor side morbidity, poor quality and lack of availability.[2,3] Synthetic grafts have previously been developed and are routinely used for large vessel grafting in patients with limited availability of autologous grafts.[4] Poor patency and thrombotic cascade initiation limit the use PTFE for small diameter applications (<6mm). [5,6] To combat these issues biohybrid scaffolds have gained momentum in the past decade – with various biocompatible materials explored as cell growth-promoting scaffolds. Biohybrid scaffolds combine the tunability and stability of synthetic materials such as poly(glycerol sebacate)-methacrylate (PGS-M) with the ability of cells to readily deposit extracellular matrix (ECM) rich in proteins such as collagen and elastin.

Materials and methods To synthesise the polymer, equimolar amounts of glycerol and sebacic acid were reacted together in a melt- polycondensation reaction resulting in poly(glycerol sebacate) as described previously by the Claeyssens group[7]. To make the polymer photocurable, PGS was functionalised through the addition of methacrylate groups. Emulsion templating was then used to produce highly porous, high internal phase emulsions (polyHIPEs) with the PGS-M polymer. The internal phase of the polyHIPE consists of water, while the polymer dissolved in toluene solvent makes up the external phase.

Results and Discussion The methacrylation of the PGS was validated through NMR analysis, and its molecular weight was calculated using gel permeation (GPC) analysis. Tubular scaffolds and disc-shaped scaffolds (for optimisation experiments) from PGS-M polyHIPEs were produced using injection moulding and cured under UV. Pore size and interconnectivity of the polyHIPEs were analysed using scanning electron microscopy. Cell selection for seeding of the tubular scaffolds will ultimately affect the collagen and elastin production, reproducibility and cost of the scaffolds. Hence, investigating if cell lines can be as efficient in the deposition of ECM with comparable composition and mechanical properties to that produced by primary cells is important. A fibroblast cell line (BJ-5TAs) will be investigated as a potential candidate for the seeding of the tubular scaffolds. ECM production will be analysed, and the collagen produced will be quantified and compared to that produced by primary smooth muscle cells. Transforming growth factor (TGF-β) will also be used to stimulate collagen production through the differentiation of fibroblasts into myofibroblasts and be compared to untreated BJ-5TAs and primary smooth muscle cells.

Conclusions The successful synthesis and subsequent functionalisation of PGS-M have been confirmed through NMR and GPC analysis. The tubular scaffolds will be cell seeded and a suitable cell line will be selected based on cell viability (resazurin) as well as collagen and elastin quantification.

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MULTICOMPONENT SUPRAMOLECULAR HYDROGELS FOR NERVE REPAI

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Introduction. Nerve repair poses a significant challenge in regenerative medicine field due to the intricate and time-sensitive nature of neural tissue regeneration.[1] Current treatment methods rely on using nerve autografts, allografts, or xenografts for larger gaps and suturing of the nerves for smaller gaps. Unfortunately, these approaches have drawbacks such as limited tissue availability, complex harvesting, scarring, insufficient nerve regeneration and neuron misguidance.[2] Hydrogel-based biomaterials are promising candidates for nerve regeneration as they present several advantages such as relatively simple and cheap manufacturing, injectability, biocompatibility and mechanical and chemical tunability.[2] Low molecular weight (LMW) hydrogels are composed of gelators that have a molecular mass of <1000 Da and exhibit the ability to self-assemble in response to application of a trigger such as pH, temperature, or solvent change. LMW hydrogelators are often composed of small building blocks such as peptides and present further advantages over regular hydrogels such as easy synthesis and functionalisation, and effective removal from the body through the renal system.[3] When developing hydrogels for nerve repair, material stiffness and conductivity are some of the most important properties to consider as both have been shown to control many neural cell processes influencing tissue regeneration such as cell migration, adherence, and proliferation.[4]
In this project hydrogels formed by two supramolecular LMW gelators, naphthalene functionalized with diphenylalanine (2-NapFF) and perylene bisimide appended with leucine (PBI-L), were studied. 2-NapFF is a well-known self-assembling compound that can be used to form hydrogels exhibiting storage modulus (G′) of ~15 kPa,[5] whereas perylene bisimide (PBI) based gelators exhibit conductive properties and ability to self- assemble, especially when functionalised with amino acids at the imide position.[6] The aim of this study is to create mechanically tunable and conductive hydrogels using multicomponent hydrogel system composed of the two supramolecular gelators.

Materials and Methods. 2-NapFF and PBI-L solutions were produced by dissolving the two gelators in phosphate buffered saline (PBS)/0.1 M NaOH solution independently. Resulting 2-NapFF and PBI-L solutions were pH adjusted using 1 M NaOH to pH 10.5 and 7.4 respectively. Supramolecular 2-NapFF and PBI-L hydrogel formation, both individually, and as a multicomponent system, was triggered by the salt addition method using $CaCl_2$. The resulting solutions were left to gel overnight. The effect of different gelator ratios on hydrogel formation were investigated using rheology with rotating vane geometry.

Results and Discussion. PBI-L hydrogels were successfully formed in PBS using the salt addition method as proved by the vial inversion and rheological tests. From the rheology results it was observed that pure PBI- L hydrogels exhibited G′ of ~1 kPa which is equivalent to some of the softest tissues in the body such as the brain.[7] In order to develop hydrogels for peripheral nerve repair, mechanical strength of the PBI-L hydrogels needs to be increased. To increase hydrogel stiffness, pre-gelled PBI-L and 2-NapFF solutions were mixed at different concentration ratios to create multicomponent composite hydrogels. PBI-L/2-NapFF composite hydrogels were successfully formed as proved by the methods used on pure PBI-L hydrogels. From the rheology results it was shown that mechanical strength of the composite gels can be tuned by varying 2-NapFF ratio within the pre-gelled PBI-L/2-NapFF solution and hydrogels exhibiting G′ values of ~30 kPa can be formed using this system.

Conclusions. PBI-L based hydrogels offer potential for nerve repair due to their conductivity and self- assembly. However, pure PBI-L gels have been noted to be very soft limiting their utilisation for nerve repair. In this study, it was shown that incorporating supramolecular gelators such as 2-NapFF can be used to create PBI-L based hydrogels with tunable mechanical properties. However, further work is required to investigate biocompatibility and conductivity of these hydrogels. Therefore, future experiments will focus on studying the effect of 2-NapFF addition on hydrogel conductivity properties and evaluation of cell viability when cultured on the composite hydrogels.

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ENGINEERING A NOVEL BONE-TENDON JUNCTION SCAFFOLD FOR TRANSPLANTATION

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Abstract theme: Bioengineered Models

Introduction:
Annually, 1.7 billion musculoskeletal injuries occur, often involving the bone-tendon junction (BTJ). Current treatment options ranging from repair to reconstructive surgery, yield suboptimal outcomes due to structural inconsistencies making the tissue engineering approach a more reliable option to achieve long term benefits.

Materials and methods:
In this study, we pioneered a comprehensive approach to address this clinical challenge. We employed a novel decellularisation process on BTJ tissue obtained from rabbit lower limbs (N=22). Demineralisation was executed over 5 days using 5% formic acid, followed by an 8-day proprietary decellularisation method. The effectiveness of the decellularisation process was confirmed through histological characterisation and DNA quantification. The decellularised bone segments were further treated by immersion in Ca-P simulated body fluid for 24 hours to promote mineralisation and enhance cell attachment. To facilitate recellularisation, adipose-derived stem cells (ADSCs) were cultured in differentiating media for 14 days, differentiating into osteoblasts and fibroblasts for the bone and tendon segments, respectively. Cell characterisation was validated through an alkaline phosphatase assay and the vimentin marker to confirm cell phenotype. Using a stepper motor device, the differentiated cells were precisely injected into their respective segments under cyclical mechanical loading conditions, simulating native tissue physiology.

Results and discussion:
DNA quantification revealed DNA content of <50ng/mg ($p<0.05$) in the bone segment. Histological analysis performed on day 5 confirmed successful recellularisation, with abundant nuclei evident within the tissue. Comparative analysis indicated similarities in properties between control bone, remineralised bone, and recellularised bone. This pioneering approach shows great promise as an effective regenerative technique, potentially bridging the current clinical gap in BTJ injury treatment.

Conclusion:
Our innovative approach offers a ray of hope for the effective treatment of BTJ injuries, promising to revolutionise clinical outcomes in this challenging domain.

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A NOVEL METHOD OF QUANTIFICATION AND VISUALISATION OF ANGIOGENESIS OF A 3D POROUS SCAFFOLDS ON EX OVO CAM MODELS

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Introduction: The efficacy of tissue engineering applications is critically dependent on the choice of biomaterials with angiogenic properties, which facilitate the development of new blood vessels from pre-existing vasculature. Angiogenesis is essential for supplying nutrients and oxygen to infiltrating cells and for the remodeling of the scaffold within the body to form new tissue. Therefore, accurately quantifying the angiogenic potential of a biomaterial post-implantation is imperative before proceeding to clinical testing. Currently CAM models are most sustainable 3D models to study angiogenesis in biomaterials as they are cost-effective, versatile and they mimic *in vivo* vascular niche. This model adheres to the principles of replacement, reduction, and refinement (3R), providing an ethical approach to investigate angiogenesis. However, they have inherent weakness such as superficial observations and quantification challenges. To address this issue, we have developed a novel approach to observe and quantify angiogenesis in biomaterials using CAM models.

Materials and Methods: In this study, biomaterials used were fibrin-elastin based scaffolds it was most angiogenetic material as per our previous in vivo studies (1). We have used the *ex ovo* chicken chorioallantoic membrane (CAM) assay to study angiogenetic potential of fibrin-elastin scaffolds (2). Angiogenesis was evaluated in two dimensions (2D) using ImageJ. Additionally, three-dimensional (3D) quantification was performed following intravascular injection of the contrast agents indocyanine green (ICG) and Microfil®, using either Micro-CT and confocal microscopy to quantify the vascular volume.

Results and Discussion: Our study highlights the shortcomings of 2D quantification methods in accurately measuring vascular volumes and points out the significant discrepancies in quantification outcomes when comparing Microfil® contrast agent to ICG dye, with the latter showing a substantial 86% increase in angiogenesis measurement ($p < 0.05$). Notably, ICG dye demonstrated superior effectiveness in staining vessels not just on the surface but throughout the entire scaffold structure, unlike Microfil®, which predominantly stained vessels at the scaffold's periphery. These findings emphasize the essential role of 3D quantification techniques for a precise assessment of angiogenic qualities in biomaterials, suggesting that ICG dye offers a more thorough analysis of angiogenesis within scaffolds.

Conclusions: This study demonstrates the limitations of 2D angiogenesis quantification and the superiority of 3D quantification methods in evaluating the angiogenic potential of biomaterials. The significant differences in vascular volume quantification between Microfil® and ICG dye emphasize the need for comprehensive 3D assessments to accurately measure angiogenic properties before clinical implementation. Our findings suggest that ICG dye is a more effective contrast agent for detailed angiogenesis evaluation in fibrin-elastin scaffolds.

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DECELLULARISATION-BY-PERFUSION: A NOVEL APPROACH TO ENGINEER > 50CM OFF THE SHELF SMALL INTESTINE GRAFT FOR TREATMENT OF INTESTINAL FAILURE

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Introduction: Intestinal failure (IF) is a chronic condition that results in the partial or complete restriction of the digestive and absorption capacity of the intestine. It can be derived from anatomical abnormalities such as short bowel syndrome or as a result of functional damage caused by other gastrointestinal disorders, namely cancers or severe Crohn's disease cases. IF patients often rely on parenteral nutrition, which is permanent in reportedly over 50% of cases (1). Only in the United Kingdom, there is an estimate of up to 21 per million population new home parenteral nutrition (HPN) cases per year (2), although it has been suggested that reported data was underestimated by approximately 30% (3). An increased trend of HPN patients has also been noted over the last decade across different European countries (3). Small intestine (SI) decellularisation can offer alternative therapeutic strategies for irreversible IF. Nevertheless, intestinal decellularisation has remained a challenge due to the size and complexity of the tissue. To date, attempts of intestinal decellularisation have been limited to small tissue fragments of up to 3 cm (4;5). We aim to address the long-term IF clinical management gap by potentially facilitating intestinal graft engineering for transplant through the decellularisation of clinically relevant (>50cm) porcine small intestine fragments.

Materials and methods: This study presents a novel dual submersion and perfusion decellularisation protocol that combines chemical, enzymatic, and physical approaches to achieve a decellularised small intestine scaffold of over 50 cm of length. Porcine tissue was obtained in-house under ethical approval, cut into 50 cm fragments, and stored at -80 degrees until use. Decellularisation of the porcine bowel tissue was carried out following a modified version of the in-house protocol adapted for a perfusion setup. Hypertonic and hypotonic solutions, followed by enzymatic and detergent solutions, were simultaneously flushed through the lumen and washed over the intestinal mesenchymal surface at a flow rate of 2L/ min. The entire decellularisation process was carried out over 4 days at 80 rpm constant rotation. Protocol performance was evaluated through DNA quantification and confirmed by H&E staining. Damage to ECM integrity was evaluated through Picro-Sirus staining.

Results & Discussion: Preliminary data reveals an average DNA concentration of 48.16 ng per mg of porcine bowel tissue (n=5), resulting in an over 30-fold decrease when compared to the control tissue average DNA concentration of 1714.06 ng/ mg (n=3). Although this reduction is insignificant (p value = 0.056, Mann-Whitney U test), it is well below the established DNA concentration threshold for decellularised tissue of 50ng/mg. H&E staining confirmed the absence of stained nuclei. Nevertheless, collagen staining showed partial but statistically non- significant damage to ECM internal structure. In fact, the crypt structure remained virtually intact when compared to the control tissue. By using this developed protocol we successfully achieved SI decellularisation in fragments of over 50 cm in size with limited tissue damage.

Conclusion: Organ-derived intestinal grafts are a promising approach to procure a long-term solution of IF complications. This protocol overcomes the current limitations in intestinal graft sizes and opens the window to engineering larger, functional, and clinically relevant SI grafts for tissue regeneration. The next steps will include bioreactor- based recellularisation with organoids to engineer fully functional SI constructs.

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DEVELOPMENT OF A 3D IN VITRO SYNOVIUM MODEL TO STUDY IMMUNE RESPONSE IN INFLAMMATORY BOWEL DISEASE

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Introduction: Inflammatory bowel disease (IBD) is a chronic intestinal inflammation and exhibits extra-intestinal manifestations, including of the joints[1], with up to 35% of patients displaying musculoskeletal disorders including spondyloarthropathies (SpAs); most notably ankylosing spondylitis (AS). AS is centralised in the synovium; a soft fibrous tissue facilitating movement of the joint, which undergoes substantial histological changes throughout inflammation[2], contributing to both synovitis and bone destruction. In the clinic, patients present with pain, reduced joint mobility and swelling. Whilst animal models are commonly used to study these diseases, they may not accurately represent human physiology. Alternatively, *in vitro* models simulate cell- pathogen interactions, offering insights into molecular pathways of disease. Three-dimensional (3D) *in vitro* models, constructed with biocompatible materials, allow for the spatial organisation of human tissues to be modelled, thus reflecting physiological conditions.

Materials & methods: Natural materials such as collagen and HA are promising candidates for *in vitro* synovium modelling. This work explores the development of a novel 3D *in vitro* model of synovium, using a collagen, hyaluronic acid (HA) and 4-arm poly(ethylene) glycol (PEG) hydrogel composition, termed CHA. Based on previous work, photocrosslinking methods have been employed to enhance the physical properties and overall stiffness of the gel, such that methacrylated-HA will instead be investigated for use within the hydrogel (CHA- M). Building on previous experiments, different CHA-M formulations have been compared, with concentrations of collagen and PEG adjusted according to the nature of the formed hydrogel. In addition, duration of UV curing of CHA-M (at 365nm) has been analysed, alongside cytotoxicity and cell viability assays. We propose to characterise CHA-M, exploring its gelation, viscoelastic properties, porosity and degradation, alongside its effects on the biological activity of IBD-AS-relevant cells, including neonatal human dermal fibroblasts, synoviocytes and macrophages.

Results & discussion: A higher ratio of collagen:PEG was found to be optimal, when considering gelation time and viscoelastic properties and was analysed within the range of 10-15mg/ml collagen and 8-12 mg/ml 4-arm PEG, with the ideal combination assessed through inverted tube tests, rheological analyses and extrusion- based testing. Gelation time via inverted tube tests demonstrated that a concentration of 10mg/ml collagen was too little, with no gelation observed, whereas 15mg/ml collagen formed a gel almost instantaneously after the addition of PEG. Real-time viscoelastic analysis using Rheolution's Elastosens-Bio revealed that the stiffness of CHA-M (at 1mg/ml HA – 15 mg/ml collagen – 12 mg/ml PEG), peaked around 10 mins of incubation at 37°C, prior to photocrosslinking, based on an average shear storage modulus (G') value of 1200 Pa and low shear loss modulus (G''). The stiffness of the gel was further increased through incubation of the gel under 365nm UV light at 30% intensity: 9.8mW/cm^2, for 5 minutes.

Conclusions: CHA-M presents as a promising material for modelling the synovium, as it is a natural material, able to be extruded and possesses biomimetic characteristics of human synovium tissue, including similar stiffness and composition. In the future, the developed formulations will be 3D printed using extrusion-based bioprinting. The hydrogel will also be combined with immune cells, including but not limited to macrophages and dermal fibroblasts, to recapitulate the cellular environment of the intimal and subintimal synovium layers.

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REACTIVE JET IMPINGEMENT (REJI) 3D PRINTING OF HIGH-DENSITY CARDIAC CULTURES

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Abstract theme: Bioengineered Models / Enabling Technologies / Biomaterials

Introduction:

Due to the limited availability of human myocardium samples and due to the difficulty associated in culturing primary cardiomyocytes, cardiovascular diseases are difficult to study as well as model in vitro. Hence the demand for the application of tissue engineering strategies to repair and model cardiac tissues have increased globally with a rise in conventional tissue engineering approaches for cardiac regenerative and diagnostic applications. A key objective for this approach is to bioengineer 3D cardiac tissue mimicking models that would facilitate the study of cardiac biomarkers for a sustained period. An ideal model should have the ability to recapitulate the hearts 3D anisotropic tissue structure, orientation of Extracellular Matrix (ECM) network, vascularization, and circulation. These conditions invitro can be achieved by engineering physiologically relevant 3D models by embedding cells in biomaterial matrices which requires methods and systems to host cells and to control cell-ECM interactions. These biomaterials will not only serve as scaffolds for tissue formation but will also provide a highly controllable microenvironment.

Materials and Methods:

Despite efficacy, current printing strategies can only print constructs with limited printing accuracy, low cell viability and density. Reactive jet impingement (ReJI) is a bioprinting process developed at Newcastle University which creates gel droplet streams through in air reaction of polymer and crosslinker solutions. Suspending cells in one or both solutions allow cell-filled-gel droplets to be created. By jetting the gel pre- cursor solutions and then producing the gel by a downstream reaction, high shear stresses are avoided, and this allows high cell density gels (up to 40 million cells/mL of gel) to be created. The process is drop-on- demand, and so is able to print gel droplets onto any substrate, including rough and delicate substrates. The key objective of this study was to bioengineer biocompatible cardiac cultures and asses their potential for use as in-vitro cardiac models. We hypothesized that a 3D bio printed cardiac construct generated via ReJI bioprinting with HL-1 cardiomyocytes would create a contractile 3D human cardiac model like tissue. Based on previous work, we used a collagen-alginate-fibrinogen (CAF) bioink with HL-1 cardiomyocytes at a density of 10 million cells/ml of gel and printed directly within well plates. Printed cardiac constructs were then biologically characterized over a period of 7 days to explore cell viability, proliferation, microstructure, cardiomyogenic gene expression of functional genes: MYH6, ANP, CONN43, calcium influx and electrical activity via multi-Electrode array (MEA) system.

Results and Discussion:

All 3D printed cardiac cultures were highly viable, proliferative and expressed cardiomyogenic specific genes throughout all time points with a dense tissue-like structure formed by day 3 itself. Calcium fluctuations were significantly increased from day 1 to day 7 and an increase in potent electrical activity was observed throughout the culture period via the MEA system.

Conclusion:

We were able to 3D bio print a highly bio-functional cardiac construct using ReJI and so this printing technique could be used as a new alternative to generate high cell density cardiac models to be used as swift delivery systems or even as laminar tissue implants for regenerative purposes.

Perfusion chamber for the investigation of microbubble oscillation in bone fractures

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Introduction: Approximately 2-10% of bone fractures result in delayed or non-union healing. The associated complications have a significant negative impact on patients' lives and surgery carries a high risk of disease transmission. Gas-filled microbubbles (MBs) potentially offer a non-invasive, ultrasound-stimulated targeted drug delivery system for bone fracture treatment. However, there is a lack of in-vitro models recapitulating the physical and geometrical properties of bone fractures, which could be employed in laboratory-scale research on novel ultrasound-mediated therapies. The aim of this study was to develop a perfusion chamber to study the behaviour of ultrasound-stimulated microbubbles.

Materials and Methods: MBs were formulated by sonication of suspensions of 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), polyoxyethylene (40) stearate (PEG(40)s) and 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine (DiI) at molar ratios of 18:2:1. Perfusion chambers were manufactured via replica moulding. The device design comprised a 2 mm wide channel passing through a series of bone fracture geometries. Positive moulds were designed in SolidWorks and 3D printed using stereolithography. Upon fabrication, moulds were baked in the oven at 120°C for 120 min and exposed to ultraviolet light for 120 min. Subsequently, the moulds were filled with liquid polydimethylsiloxane (PDMS, Sylgard 184, 5:1 ratio between silicone elastomer and curing agent) and were then left to cure overnight. The solidified PDMS layer containing the channel features was then bonded to another layer of PDMS. MBs were perfused through two systems, after its placement in custom designed ultrasound stimulation chambers. One employed for the in-vivo testing with a passive cavitation detection (PCD) device placed in the center of a transducer (operating at 0.74 MPa, 500 kHz frequency and duty cycle of 1%) and the other for in-vitro testing with a PCD device placed on the opposite side of a transducer (operating at 1.0 MPa, 1000 kHz frequency and duty cycle of 30%) [1]. MBs concentration in the in-vitro operating system varied within the range of 1.271×10^6 to 1.27×10^7 MBs/mL and in the in-vivo system from 1.13×10^6 to 1.13×10^8. PCD device and TiePie were used to detect and record acoustic emissions. COMSOL 6.1 with frequency-domain simulations were carried out to model the ultrasound pressure field within the device, as a function of bone geometry.

Results and discussion: The total power of the acoustic emissions generated by MBs under ultrasound stimulation increased significantly with respect to MB concentration ($P<0.001$). In the static experiment, the total acoustic emissions power decreased with respect to time due to MB destruction. COMSOL simulations showed increased acoustic pressure magnitude in the focus point of the transducer (placement of MBs channel) from 0.97 MPa to 1.41 MPa when the fracture gap width was set to 0.5 mm and 4.0 mm, respectively. Fracture gap simulations predicted higher peak pressures in the whole volume of in-vitro system, located out of the focus zone of the transducer, reaching 2.28 MPa for the 0.5 mm fracture model. Physical obstruction created by a narrow fracture gap below 1.5 mm, reduces ultrasound pressure field in the transducer focus. This is a very important finding for selecting appropriate MB concentrations and US parameters for specific fracture geometries.

Conclusions: As would be expected the concentration of MBs has a significant impact on their activity upon ultrasound stimulation in a non-obstructed system. We also found that increased ultrasound pressure caused by the bone geometry has a high impact on MB cavitation due to lower acoustic pressure, and it could potentially impact MB detection and biological effects such as sonoporation, due to high pressure generated on the side of the transducer by reflected ultrasound waves.

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NOVEL COMPOSITES FOR COMBINED DENTAL PULP CAPPING AND TOOTH RESTORATION

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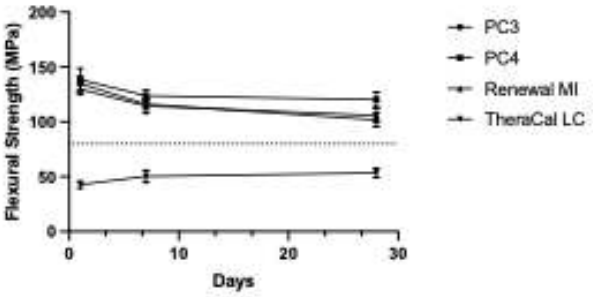
Abstract theme: Biomaterials

Introduction Currently, when a carious lesion progresses to a stage where the dental pulp is exposed, materials such as TheraCal LC are used as a protective layer prior to tooth restoration. Tooth restoration also requires multiple steps. Renewal MI has been recently developed to enable reduced caries excavation and single-step placement. However, its compatibility with pulp cells has not yet been studied. In this work, the Renewal MI has been modified to determine how the materials are compatible with pulp cells, aiming to replace pulp capping materials and simplify dental procedures. The objective of this study is to develop novel materials with suitable chemical, physical, and biological properties, enabling them to be simultaneous pulp capping and tooth restoration materials.

Materials and Methods The materials were prepared by mixing UDMA (72%), PPGDMA (24%), and 4-META (3%) monomers with CQ as a photo initiator (1%). Then they were combined with 75 wt% fillers containing MCPM and $Ca(OH)^2$ at varying ratios. Setting kinetics were assessed by FTIR spectroscopy. Mechanical strengths were tested using a Universal testing machine after being stored in simulated body fluid for up to 1 months. Then the mineral precipitation was determined using SEM. The mass and volume changes, and acidity, were observed for 1 month in water storage. The pulp cell compatibility was investigated by CCK-8 and Live-Dead imaging assays. The investigation included the surface of materials as well as the extracts from both cured and uncured materials.

Results The addition of $Ca(OH)^2$ to the formulations resulted in a higher reaction rate of polymerization and a greater degree of monomer conversion, compared to Renewal MI and TheraCal LC. The flexural strength demonstrated in (a) exhibited a substantial higher compared to TheraCal LC. In addition, the modified formulation presented increased alkalinity with low level of mass and volume changes. Furthermore, the formulations demonstrated a significant level of compatibility when growing cells directly on the cured materials and in uncured extracts. Although the uncured-material extracts indicated reduced cell compatibility, the level of cell compatibility of modified formulations and Renewal MI was still considerably greater than that of TheraCal LC.



Discussion

$Ca(OH)^2$, widely accepted and used in pulp capping treatment, derives advantages from its ability to release calcium and hydroxyl ions. These factors play a crucial role in pulp cell differentiation, a key aspect for pulp capping treatment. Renewal MI encountered regulatory issues with PolyLysine as reactive fillers; therefore, $Ca(OH)^2$ was incorporated into the formulations. Additionally, the calcium and phosphate ions released from $Ca(OH)^2$ and MCPM will enhance the remineralising abilities. Achieving a high level of monomer conversion is necessary for ensuring cell compatibility and obtaining desirable mechanical properties. Clinically, the rapid setting of materials also decreases cytotoxicity, as the duration of contact between pulp cells and uncured materials is very brief. Furthermore, the uncured pastes exhibited a very low level of cytotoxicity. In the long term, cured materials would not have an adverse effect on the pulp cells due to their remarkable high cell compatibility and relatively neutral pH. Conversely, the low degree of monomer conversion in TheraCal LC, along with its significant increase in mass and volume due to water absorption, led to a decrease in its mechanical properties. Moreover, had a detrimental impact on the vitality of the pulp cells. The excessive water expansion will restrict its application to only a thin layer. Except for TheraCal LC, all experimental formulations exceeded the ISO4049 minimum requirement of flexural strength of 80 MPa for tooth restoration materials.

Conclusions The modified formulations containing $Ca(OH)^2$ and Renewal MI composites possess the potential for novel simultaneous pulp capping and tooth restoration materials.

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AUTOMATED BIOFABRICATION OF BIOMIMETIC GLAUCOMA *IN VITRO* MODELS

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Corresponding author: hcl954@student.bham.ac.uk– PhD student (final year) **Abstract theme:** Bioengineered Models

Introduction

Primary open angle glaucoma (POAG) is currently the leading cause of irreversible blindness. POAG pathogenesis originates from the trabecular meshwork (TM) tissue, which becomes fibrotic due to trabecular meshwork cell (TMC) dysfunction. However, the development of an *in vitro* model that can induce *in vivo*-like behavior, or as to what stimuli modulate TMC phenotype, remains elusive. This study elucidates biophysical and biochemical spatial cues that induce *in* vivo-like TMC phenotypic states in both healthy and POAG scenarios through 3D *in vitro* modelling. Automated models were produced, creating a biomimetic TM *in vitro* model with tunable collagen properties, targeted towards high-throughput screening (HTS).

Materials and Methods

The gel-aspiration-ejection method (GAE) was utilized for the development and optimization of a high- throughput drug screening platform. Initially, variations in collagen fibre parameters were evaluated through tensile testing, SEM/micro-CT imaging and collagen fibre density (CFD) quantification. Human TMC-laden collagen hydrogels (250,000 cells/mL) were subjected to the GAE within a 96-well plate. To induce POAG attributes, 5ng/ml of transforming growth factor-beta2 (TGF-β2 (PODS® technology)) was integrated into PC- collagen. Thereafter, TMC phenotypic differences were assessed; immunocytochemical staining (elastin deposition, fibronectin, alpha-SMA, α-β-crystallin) and metabolic capacity was sustained up to 2-weeks (resazurin reduction). The HTS developed was further tested with varying concentration of ophthalmic drug candidate, Netarsudil, compared to 2D TMC cultures. Efficacy of the drug candidate was compared to through variations ECM remodeling enzymes, MMP-2/TIMP-2 ratios (ELISA), yes-associated protein (YAP) translocation, cytoskeletal remodeling and differences in fibrotic genetic markers.

Results and Discussion

Optimised collagen parameters produced heightened collagen anisotropy and CFD (7.33±1.01%) compared to gold standard hydrogels (2.07±0.58%). Thereafter, TMC expressed relevant *in vivo* markers 3- days post incubation in both native and POAG-like *in vitro* models (elastin deposition, fibronectin, alpha-SMA, α-β-crystallin) with maintained TMC alignment and sustained, 2-week metabolic activity compared to control.

Optimal Netarsudil efficacy was initially determined by quantifying variations in physiologically relevant MMP-2/TIMP-2 protein ratios. As decreased MMP-2/TIMP-2 ratios are associated with POAG, enhanced drug- induced alterations were consistent within all 3D cell culture donor samples. Notably, optimal drug concentrations varied between donor and TGFβ-2 presence, with 0.01-0.1μM Netrasudil producing a therapeutic response. YAP translocation was noted in 3D compared to 2D TMC cultures, with marked difference in cellular morphology and varying degrees of actin filament breakdown when introducing varying drug candidates.

Conclusion

A biomimetic 3D *in vitro* model of the TM was optimized, producing an initial panel of positive *in vivo* markers that was dependent on collagen structural framework or/and TGF-β2 (biophysical/biochemical spatial cues). Once established, biophysical parameters were reproduced in an automated manner for future high- throughput screening. The formation of the 3D TM *in vitro* models provided a more accurate response to ophthalmic drug candidates for HTS compared to the 2D gold standard.

INVESTIGATING THE IMMUNOMODULATORY AND ANTI-CANCER EFFECTS OF ACEMANNAN IN ACUTE MYELOID LEUKEMIA

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Introduction The bleak prognosis of acute myeloid leukaemia (AML) is partly due to chemoresistance, which arises from the complex interaction between cancer cells, stromal cells, and the surrounding microenvironment. Three key cell types – bone marrow mesenchymal stem cells (BMSCs), macrophages, and AML cells – contribute to AML progression: AML cells transform the stromal cells, resulting in BMSCs aiding chemoresistance, while macrophages transform from anti-cancer to AML-supporting phenotype[1,2]. Developing 3D models that mimic the disease niche is vital to support more representative cancer research. Since MSCs and macrophages are mainly localised within the perivascular and central marrow niches, softer matrices between 0.3-20 kPa are desirable.

Acemannan has traditionally been used to treat skin ailments. More recently, pharmacological studies have reported pro-haematopoietic and anti-cancer effects[3,4]. Various formulations of acemannan have been reported to polarise macrophages towards a pro-inflammatory macrophage phenotype, exerting immunomodulatory, anti-cancer effects[5,6]. Acemannan has been noted to have an anti-cancer effect against fibrosarcoma and adjuvant effects in a mouse cervical cancer model, mediated by macrophages releasing IL- 1, interferon, and TNF-α[7,8]. However, the mechanism of action and effectiveness of acemannan as an anti- cancer compound is poorly understood, with limited research suggesting a link to macrophage polarisation in its anti-cancer effects. Additionally, its potential application in AML remains unstudied.

Materials and Methods A novel collagen hydrogel mimicking the BMME was developed to explore the effects of acemannan on AML cells. This study used collagen hydrogels obtained via Type I rat tail collagen fibrillogenesis, containing varying concentrations of acemannan (0, 1, and 2 mg/mL). Human BMSCs and a human AML cell line, MOLM-13, were encapsulated in these collagen hydrogels in monoculture and co-cultures. Cell viability was assessed with and without cytarabine treatment to study the potential anti-cancer and adjuvant effects of acemannan and the chemoprotective effects of MSCs on MOLM-13.

Results and Discussion Firstly, high viability and cell retention of MOLM-13 and BMSCs were confirmed in the control 0 mg/mL collagen hydrogels, supporting the use of our hydrogel system as a BMME model. Our results show that acemannan decreases MOLM-13 cell count in mono- and co-cultures while increasing BMSC cell count. When MOLM-13 is co-cultured with BMSCs, acemannan was noted to accelerate the anti-cancer effect of cytarabine and reduce MOLM-13 cell count without cytarabine treatment.

Conclusion These findings suggest acemannan has anti-leukaemia effects while increasing BMSC cell counts. Acemannan also appears to exert a synergistic effect with cytarabine against MOLM-13. Further investigations, including macrophages in our BMME model, could improve our understanding of how acemannan exerts its anti-cancer and adjuvant effects in AML.

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3D BIOPRINTING TISSUE ENGINEERED MENISCAL CONSTRUCTS

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Abstract theme: Bioengineered Models / CDT Session

Introduction

Meniscal injuries affect over 1.5 million people across Europe and the USA annually. Injury greatly reduces knee joint mobility and quality of life and frequently leads to the development of osteoarthritis. Tissue engineered strategies have emerged in response to a lack of viable treatments for meniscal pathologies. However, to date, constructs mimicking the structural and functional organisation of native tissue, whilst promoting deposition of new extracellular matrix, remains a bottleneck in meniscal repair. 3D bioprinting allows for deposition and patterning of biological materials with high spatial resolution. This project aims to develop a biomimetic 3D bioprinted meniscal substitute.

Methodology

Meniscal tissue was characterised to effectively inform the design of biomaterials for bioprinting constructs with appropriate structural and functional properties. Histology, gene expression and mass spectrometry were performed on native tissue to investigate tissue architecture, matrix components, cell populations and protein expression regionally across the meniscus. 3D laser scanning and magnetic resonance imaging were employed to acquire the external geometrical information prior to fabrication of a 3D printed meniscus. Bioink suitability was investigated through regional meniscal cell encapsulation in blended hydrogels, with the incorporation of growth factors and assessed for their suitability through rheology, scanning electron microscopy, histology and gene expression analysis. Bioprinting of 3D meniscal constructs were fabricated through zonal deposition of regionally tailored bioinks.

Results

Meniscal tissue characterisation revealed regional variations in matrix compositions, cellular populations and protein expression. The process of imaging through to 3D printing highlighted the capability of producing a construct that accurately replicated meniscal geometries. Regional meniscal cell encapsulation into hydrogels revealed a recovery in cell phenotype, with the incorporation of growth factors into the bioink's stimulating cellular re-differentiation and improved zonal functionality. Bioprinting of bioinks regionally enabled the fabrication of a 3D meniscal construct with regional variations in cell and matrix deposition.

Conclusions

Meniscus biofabrication highlights the potential to print patient specific, customisable meniscal implants. Achieving zonally distinct variations in cell and matrix deposition highlights the ability to fabricate a highly complex tissue engineered construct.

DEVELOPMENT OF AN EXPERIMENTALLY TRACTABLE IN VITRO HUMAN MODEL OF OSTEOGENESIS

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Introduction Osteogenesis is a complex biological phenomenon crucial for skeletal development, maintenance, and repair. Understanding the underlying mechanisms of osteogenesis is vital not only for unravelling fundamental aspects of musculoskeletal biology but also for advancing clinical treatments for bone-related disorders. To achieve this understanding, researchers often rely on in vitro models that simplify and elucidate key processes. One particularly valuable model of osteogenesis is the differentiation of mesenchymal stem cells (MSCs) into osteoblasts, the cells responsible for bone formation. MSCs are found in bone marrow and can differentiate into skeletal cell types, including osteoblasts. However, primary MSCs are highly heterogeneous, short-lived, and routinely grow in 10% foetal bovine serum (FBS), which contains over 2000 undefined molecules; this limits experimental consistency and long-term use for research and therapeutic purposes. To address these challenges, we generated and characterised a series of immortalised human clonal MSC lines. One of those cell lines, named Y101, favoured osteogenic differentiation over chondrogenesis and adipogenesis. Here we determined how the Y101 MSC line may be developed as an experimentally tractable model of osteogenesis.

Materials and Methods Y101 cells were weaned from 10% FBS to 0.5% FBS over 20 weeks to establish a low-serum adapted cell line termed Y101.5 and RNA-Seq was used for transcriptomic profiling. Y101 and Y101.5 cells were evaluated for their osteogenic capacity using in vitro differentiation assays for up to 21 days in the presence and absence of dexamethasone (Dex) and FBS. Alkaline phosphatase (ALP) levels were measured both at baseline and during differentiation using the para-nitrophenyl phosphate (pNPP) assay. Mineralisation was assessed using alizarin red and von Kossa staining techniques. Protein expression of osteogenic markers was analysed by western blotting.

Results and Discussion RNA-Seq identified 510 differentially regulated transcripts in Y101.5 versus Y101, with upregulated pathways related to fatty acid/lipid/cholesterol metabolism. Y101 and Y101.5 MSCs exhibited distinct osteogenic differentiation profiles, notably with Y101.5 cells showing accelerated mineralisation compared to Y101 cells. Alizarin red elution demonstrated higher calcium deposition in Y101.5 cells at day 7 compared to Y101 cells at day 14, as assessed by spectrophotometry. Similarly, phosphate deposition, as visualised by von Kossa staining, followed a pattern consistent with the alizarin red findings. The pNPP assay revealed basal ALP activity at day 0 to be approximately 4 times higher in Y101.5 cells compared to Y101 cells, with increasing activity for both cell types at later timepoints. This innately elevated ALP activity may partially contribute to explaining the accelerated osteogenic differentiation profile of the Y101.5 cells over the Y101 cells. Dex is a glucocorticoid with pleiotropic effects, which is routinely used as a non-physiological stimulus for osteoblastic differentiation in vitro. Paradoxically, long-term glucocorticoid exposure causes bone loss. We demonstrated that Y101.5 cells maintained an accelerated osteogenic profile in the presence and absence of Dex, pointing to an osteolineage predisposition with the ability to differentiate independently of Dex. Moreover, in the absence of FBS and Dex, Y101.5 cells continued to exhibit robust accelerated mineralisation (calcium and phosphate deposition) versus Y101 cells at day 7. Western blot analysis showed higher ALP expression in Y101.5 compared to Y101 at basal levels at day 0, with elevated expression levels at later timepoints, which was supported by pNPP activity assays. Interestingly, the expression of Runt-related transcription factor 2 (RUNX2), a critical regulator of osteogenesis, was consistently detected at equivalent levels in both Y101 and Y101.5 across early timepoints (days 1, 2, 3, 4, and 7), indicating that enhanced osteogenesis in the Y101.5 model was not associated with increased RUNX2 expression.

Conclusions The accelerated osteogenic differentiation observed in Y101.5 MSCs in the absence of Dex and FBS, accompanied by elevated basal levels of ALP and RUNX2 expression, identifies a pro-osteoblastic phenotype. Y101.5 MSCs can serve as a defined, consistent, and reproducible model for investigating the underlying mechanisms of osteogenesis and disorders associated with pathological bone loss.

MIMICKING DOPAMINERGIC NEURODEGENERATION IN A HUMAN 3D *IN VITRO* MODEL OF PARKINSON'S DISEASE

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Introduction One of the main factors limiting the clinical translation of Parkinson's disease (PD) treatments is the absence of relevant preclinical models that can accurately predict human cellular and tissue responses to new therapeutic approaches. While animal models continue to be the gold-standard in preclinical research, they are often not representative of human disease pathophysiology[1,2]. Thus, *in vitro* human PD models may be a useful approach to assess human-specific disease mechanisms and susceptibilities to new treatment candidates. However, conventional human cellular models that rely on monolayer (2D) culture systems can fail to represent key aspects of the native microenvironment of the human brain[3]. Recent efforts have been focused on developing 3D culture systems that better mimic the spatial and mechanical properties of the brain, bringing *in vitro* cell behaviour closer to their physiological phenotype and function. In this work, a 3D hydrogel- based model of dopaminergic neurodegeneration was developed to reproduce key features of PD *in vitro* and investigate potential treatment solutions.

Materials and Methods Human neuroblastoma SH-SY5Y cells were first grown in monolayer, differentiated with retinoic acid (RA) for 7 days, and seeded within soft collagen gels. Cell viability was evaluated subsequently after 1 and 6 days using a live/dead dye exclusion assay. The expression of dopaminergic markers in SH-SY5Y cells was further induced by cultivating cellular collagen hydrogels with a neurotrophin cocktail and assessed by immunofluorescence. Finally, neurodegeneration was triggered *in vitro* by treating cellular hydrogels with 6- hydroxydopamine (6-OHDA), after which cell metabolic activity was quantified.

Results and Discussion Over 70% of RA-differentiated SH-SY5Y cells remained viable throughout the 6-day hydrogel culture period, with no significant differences between days 1 and 6. Following differentiation with the neurotrophin cocktail, SH-SY5Y cells expressed the neuronal marker βIII-tubulin and the dopaminergic markers tyrosine hydroxylase (TH) and dopamine transporter (DAT). Administration of 6-OHDA caused a marked decrease in cell metabolic activity 72 hours after neurotoxin treatment, showing that this model can potentially replicate dopaminergic neurodegeneration *in vitro*.

Conclusions 3D collagen gels can support SH-SY5Y cell survival, differentiation into dopaminergic-like phenotypes, and degeneration following 6-OHDA treatment. Future work will involve replacing this cell line with human induced pluripotent stem cell (hiPSC)-derived dopaminergic neurons and midbrain organoids. Using hiPSC lines expressing PD-associated genetic variants will allow the recapitulation of specific PD features *in vitro*, allowing the investigation of the pathological mechanisms that underlie this condition and the potential identification of new therapeutic targets.

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OPTIMISED ANIMAL-FREE GROWTH FACTORS FOR REPRODUCIBLE STEM CELL AND ORGANOID CULTURES

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Introduction Advancements in robust *in vitro* tissue-mimicking models have harnessed soluble growth factors, extracellular matrices (ECMs), and other cues, leading to the evolution of more representative models such as stem cells and organoids[1-3]. In response to the demand for reproducible, better translatable, and ethical solutions, scientists are seeking innovative, animal-free alternatives for predictive platforms, clinical applications, and expanding transformative stem cell research. Recent legislative acts promoting non-animal methodologies further underscore the challenge of obtaining ethical approval for animal-derived proteins in the future[4].

Challenge Integral to the culture of various organoids and directed stem cell differentiation are key components such as R-spondins, BMP inhibitors and FGF family of proteins. For instance, in intestinal organoids, a combination of R-spondin 1 to enhance Wnt-3a signaling and noggin for BMP family protein inhibition is commonly employed [5]. However, producing these complex extracellular proteins recombinantly presents challenges such as low yield and bioactivity preservation. Critically, production in mammalian cell expression systems is also expensive and there is risk of contamination with related endogenous mammalian proteins leading to reproducibility issues and off-target results.

Innovation Our novel animal-free recombinant proteins provide a viable alternative to address these challenges. Leveraging proprietary production processes and protein engineering technology, we have developed optimised animal-free versions of R-spondin 1, gremlin 1, the natural BMP inhibitor in the intestine and thermostable FGF-2, achieving high yield and homogeneity in *E. coli* expression systems.

Results and conclusion Our optimised R-spondin 1 supports intestinal organoids in culture at comparable or even lower molar concentrations than animal cell-derived equivalents. Furthermore, combining optimised animal-free R-spondin 1 with our engineered form of gremlin presents opportunities to enhance the scale-up of intestinal organoid cultures for applications in drug screening and disease modeling. Collaborative research conducted at Cold Spring Harbor Laboratory demonstrates how conditioned media can be replaced with recombinant R-spondin 1 in organoid cultures, indicating the potential for utilising enhanced animal-free growth factors for prolonged stem cell and organoid cultures. Additionally, our developed stabilised FGF-2 variants, such as FGF2-G3, through protein engineering showed improved reproducibility and homogeneity of long-term stem cell and organoid cultures. Unlike wild-type FGF-2 with its short half-life, FGF2-G3 offers enhanced thermostability, ensuring consistent activity over prolonged periods, thus minimising variability and promoting homogeneity in cultures.

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DEVELOPING IN VITRO 3D SYSTEMS TO STUDY GUT FUNCTION AND IMMUNITY

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Background: The gastrointestinal tract serves not only as the site for food digestion but also as a pivotal player in immunity. Dysregulation of the gut barrier function contributes to various intestinal diseases and has far- reaching consequences on the functionality of distal tissues. Notably, experimental models of systemic inflammatory disease have suggested that intestinal alterations, encompassing impaired gut barrier integrity, may affect disease pathogenesis.

Methods: We employed 3D rigid scaffolds to facilitate the co-culturing of distinct cell lines, comprising intestinal enterocytes, goblet cells, and intestinal fibroblasts. Enterocytes and goblet cells, typified by Caco-2 and HT- 29 cells respectively, were cultured at the top of the scaffold, delineated from the underlying fibroblast compartment by a collagen layer. Cell morphology and organisation were scrutinised through hematoxylin/eosin and periodic acid-Schiff (PAS) staining. Functionality was evaluated via trans-epithelial electrical resistance (TEER) and permeability studies. Lectin staining was employed to discern the expression of glycans within intestinal mucins.

Results: Our 3D system prompted the formation of finger-like structures within the Caco-2 cell monolayer, a phenomenon akin to the in vivo intestinal epithelium. Importantly, the presence of fibroblasts was indispensable for orchestrating this structural feature. Additionally, the network between fibroblasts and the epithelial layer was found to influence the glycosylation profile of the model and the overall integrity of the epithelium. Finally, we subjected the model to stimulation with the proinflammatory cytokine tumour necrosis factor-alpha (TNF) to assess its utility as an intestinal disease model. TNF up-regulated interleukin-6 (IL-6) expression while concurrently attenuating TEER potential.

Conclusions: This 3D model recapitulates the intricate characteristics of the native gut environment. Notably, it sheds light on the interplay among intestinal enterocytes, goblet cells, and intestinal fibroblasts during the formation of the gut barrier. These findings underscore the model's potential as a robust platform for studying intestinal diseases and related immune responses within a controlled laboratory setting.

NOVEL METHOD TO CREATE TUBULAR PROTEIN-BASED HYDROGELS FOR TISSUE ENGINEERING

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Introduction: The main objective of tissue engineering is to closely mimic the anatomy and physiology of tissues in our body. Various techniques, such as bioprinting and electrospinning, have been employed to generate scaffolds for 3D cell culture to recapitulate tissues and organs. However, to efficiently stimulate the cellular processes, there is a need for scaffolds that incorporate multi-functionality. We introduce a new class of protein-based hydrogels that are crosslinked using the SpyTag (ST)/SpyCatcher (SC) peptide/protein pair. The SC-ST hydrogels are genetically programmable and the covalently cross-linked hydrogel forms spontaneously upon simple mixing of the two protein components. This spontaneous gelation is well-suited for fabricating tubular-shaped gels using a novel technique called rotational internal flow layer engineering[1] (RIFLE). This technology creates a thin film of liquid on the inside of a rotating mould and we use it to produce tubular hydrogels of varying length, diameter, and thickness, thereby building multi-layered tissue-like structures.

Materials and Methods: The SC and ST proteins are expressed in *E.coli* BL21 and His-tagged proteins are purified by affinity chromatography using ÄKTA. Expression and purification have been optimized to increase protein yields per litre of culture. These proteins are then resuspended at particular molar concentrations at which gelation is achieved upon mixing. The resuspended proteins are then subjected to RIFLE by the addition of equal volumes of SC and ST to form the first layer. Gelation is allowed to occur before the next layer is added. Subsequent layers are added to build a tubular gel containing 25 layers in total. Tubes of varying thickness and length can be made by changing the rpm of the rotating mould.

Results and Discussion: Optimized expression and purification of the recombinant SC and ST proteins resulted in high yields of ~90 mg/L of culture. Tubular protein-based hydrogels were successfully made using RIFLE and examples of tubes will be shown. Encapsulation of different cells into this biofabrication system can anatomically mimic layered tissues, such as vascular, tracheal, intestinal, and ureteric. In addition, by cutting open such tubes, sheets of defined thickness can be readily made, with potential applications in dermal tissue engineering.

Conclusion: This study reveals a novel method to fabricate tubular hydrogels as a scaffold for 3D cell culture. We aim to direct this technique towards ureter tissue engineering to encapsulate different cell types in different layers, thereby creating a hydrogel with heterogenous stratification.

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N-LINKED GLYCOSYLATION IN TRIPLE-NEGATIVE BREAST CANCER AND CHROMOSOMAL INSTABILITY STUDIES IN 2D AND 3D MODELS.

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Glycosylation is one of the most important post-translational modifications, with most human proteins being glycosylated. There is increasing evidence that abnormal glycosylation can impact biological functions and protein folding. In Cancer, changes in the glycocalyx can promote invasion and metastasis. There has been extensive research on glycosylation changes in cancer. The study presented here in 2D represents the investigation of N-linked studies of four treated immortalized breast cancer cell lines MCF-7, SKBR3 (HER2+), MDA-MB-231, and BT-549 with glycosidase inhibitors Deoxynojirimycin (DNJ), and Nobel compounds of iminosugars 25,42,44 from the company Phytoquest, exposed for 24 hours. In 3D models, MCF-7 and MDA- MB-231tumoroids were fabricated and exposed to glycosidase inhibitors for 10 days, causing changes in cellularity and, therefore, reducing the number of cells in the tumoroids. The effect of glycosidase inhibitors altered cellular metabolism and responsiveness to chemotherapeutic drugs such as Doxorubicin, thus reducing cancer cell numbers and changes in the glycocalyx. Synergistic interaction between the chemotherapeutic drug Doxorubicin and glycosidase inhibitors after exposure to MCF-7 cell lines leads to the highest reduction of the treated cell lines in 2D. It is expected that a better understanding of the molecular mechanisms of tumor- associated glycans will lead to the discovery of new biomarkers.

NANOVIBRATIONAL CONTROL OF CHONDROGENIC DIFFERENTIATION

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IntroductionThe World Health Organization (WHO) has ranked osteoarthritis (OA) as the second-greatest cause of disability and the primary health disease with the fastest rate of growth [1]. OA is linked to an ageing-related breakdown of the homeostatic balance between the mechanisms that degrade and repair articular cartilage (AC) [2]. The most recent treatments focus on cell-based therapies, which are still in the research or preclinical stages and utilize biomaterials that have been seeded with chondrocytes, progenitor cells, and/or chondrogenic factors [3]. All these approaches use the transforming growth factor β (TGFβ) in cell cultures, which results in a deep cartilage/fibrocartilage phenotype (collagen X rich), rather than the smooth articular cartilage (collage II rich) that exists in vivo [4]. Therefore, novel techniques in tissue engineering (TE) can be used as promising tools for cartilage regeneration. In this new project, we have hypothesized that biophysical and biomechanical cues can be used to provide and support a clinically important chondrocyte phenotype. Here, we use bioreactors that were repurposed to deliver tiny, nanoscale vibrations into bone marrow mesenchymal stromal cells (BM-MSCs) to produce chondrocytes without the use of additional chondrogenic factors (e.g., TGFβ3) [5]. At the same time, we are trying to establish an optimal cell seeding density as several studies have shown situations in which cell to cell interactions between stem cells were associated with a stronger chondrogenic response [6]. In further research, we will turn to encapsulating the nanovibrated chondrocytes in hydrogels, where we will attempt to adjust the biophysical parameters to mimic the rigidity of in vivo cartilage.

Materials and Methods To establish an optimal chondrogenic differentiation protocol, the aggregated stem cells were seeded in different cell densities to create the micromass formation. Stro-1 selected MSCs have been seeded in $5x10^4$, $1x10x^5$and $2.5x10^4$density in different adjustable amplitudes from 15, 30, or 60 nm in 1000 Hz. Following 21 days of incubation, qPCR transcript analysis was used to quantify chondrogenic markers SOX9, ACAN, COLII & COLX expression. Furthermore, western blotting assay and immunofluorescence microscopy were used to observe and quantify the protein levels of the same chondrogenic markers. In addition, histology staining (Safranin O) was followed to observe the cartilage formation. The stiffness of the micromasses also were determined by using nanoindentation technique.

Results and Discussion Our results show that the expression of the chondrogenic markers is correlated with sociodemographic (age, sex). Younger patients showed more response to nanovibrations for differentiation into articular factors chondrocytes in 30nm amplitude after cell seeding $2.5x10^4$in contrast with the older patients. SuperPlots of gene expression indicate that COLII is more highly expressed in NK and NKCH, whereas COLX is expressed lower in NK conditions. Immunofluorescence showed an increase in ACAN expression in NK condition. The nanoindentation showed that the stiffness of the NK micromasses were significantly lower than the TGFβ containing chondrogenic media conditions, but significantly higher than control (only basal DMEM media), which indicates that the NK resulting in a positive hyaline cartilage without the hypertrophic formation.

Conclusions The goal of this project is to explore the responses of MSCs derived from patients with OA, in response to nanoscale vibration. We aim to unlock the pathway that is regulated in response to this extra biophysical stimulation, and through this pathway or a combination of different signalling pathways to upregulate the hyaline chondrogenic markers. So far, we believe that the NK shows positive data for cartilage regeneration, and we aim in the future to embed them in viscoelastic hydrogels to maintain their chondrogenic differentiation.

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HORMONE-RESPONSIVE, PATIENT-DERIVED MODELS OF THE UTERINE WALL IN A MICROFLUIDIC ARRAY

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Introduction Despite their prevalence and their profound impact on patient's lives, chronic gynaecological disorders (such as endometriosis and adenomyosis) remain under-researched. Both to advance our understanding of the cellular behaviours underlying these conditions and to provide new tools for screening of potential therapies, advanced *in vitro* models are required. 3D patient-derived cultures that mimic the uterine wall, comprising both endometrial and myometrial cell types, are an attractive and unexplored route. Here, we describe an organ- on-a-chip approach to creating such cultures, demonstrating multiplexed, hormone-responsive uterine models within a microfluidic array.

Materials and Methods Endometrial (obtained from pipelle biopsies) and myometrial (from patients undergoing elective C-sections) tissue samples were enzymatically dissociated to produce epithelial, stromal and myometrial cell fractions [1]. These cell suspensions were sequentially seeded into a microfluidic device with multiple, individually addressable channels, each containing a 5x5 microwell array. The devices were fabricated in PDMS and functionalised to create a non-adherent surface as previously published [2]. The resultant 3D cultures were maintained *in vitro* for up to 15 days and were subjected to hormone stimulation with β-estradiol (E2, 10 nM), medroxyprogesterone acetate (MPA, 100 nM) and cAMP (500 µM). Cellular organisation was monitored by live-cell imaging using fluorescent tracers and by end-point immunocytochemistry (pan-cytokeratin for epithelial cells, calponin for myocytes (SMCs), vimentin for stromal cells), with model functionality assessed by measuring IGFBP-1 and osteopontin secretion (by ELISA) following hormone stimulation.

Results and Discussion A series of cell injection protocols, sequentially seeding the three cellular fractions over 2-4 days, were investigated. Regardless of protocol, epithelial cells ultimately localised to the outer periphery of the 3D cultures. Optimum results – where self-organisation resulted in robust 3D cultures with an outer epithelial layer encircling an inner myometrial core, thus mimicking the architecture of the uterine wall – were obtained when the myometrial cells were seeded last and when 5% (v/v) Matrigel® was incorporated into the culture media. Consistent cellular organisation was obtained when directly comparing patient-derived cultures produced from different endometrial biopsies (n=3, all biopsies from secretory phase tissue). Exposure of these cultures to E2, MPA and cAMP resulted in an increase in the secretion of both osteopontin and IGFBP-1, indicating stromal decidualisation [3].

Conclusions These functional, 3D multicellular cultures show promise for personalised modelling of gynaecological disorders, enabling screening of patient-derived cultures in a medium-throughput format.

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REPRESENTATIVE PRECLINICAL MODELS OF THE HUMAN TESTIS

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Introduction:

Infertility affects approximately 8-12% of couples, with male infertility identified as the primary cause in between 10 to 50% of cases Genetic disorders leading to defects in sperm production and delivery contribute to a number of male infertility cases. Furthermore, a reported 50% decline in sperm count over the past 50 years highlights potential environmental influences on infertility [1]. However, the mechanisms behind many forms of male infertility are not fully explored and a model that accurately mimics the complex testicular microenvironment is required to improve our understanding.

Objective:

The reproductive tract is a dynamic environment, constantly undergoing morphological changes in response to hormonal cues [2]. Therefore, a comprehensive understanding of the shape, architecture, arrangement, and tortuosity of seminiferous tubules, as well as the effects of dynamic morphological changes on Sertoli cells, peritubular myoid cells, Leydig cells, and germ cells, would shed light on testicular function and its dysregulation leading to infertility. One of the primary approaches employed to study these characteristics is biomimetic testicular tissue reconstruction, utilizing tissue-engineering principles and methodologies [3]. This strategy aims to mimic the testicular microenvironment, replicating physiological conditions. This approach hopes to facilitate the maintenance of male gametes in culture, allowing for longitudinal assessment of male fertility.

Methodology:

A photocrosslinkable scaffold composed of testicular extracellular matrix (ECM), blended with methacrylated gelatin, is created to mimic the rheological properties of native testicular tissue (characterised in a separate study). Raman spectroscopy is utilized to compare the ECM scaffold composition with the native tissue. Furthermore, dissociated testicular cells will be encapsulated within the tubular scaffold and organized into multiple layers to emulate the structure of the entire testis as a 3D system. Subsequently, the impact of scaffold topography, shape, and tortuosity on testicular cell behaviour will be evaluated.

Conclusion:

The study highlights tissue property dynamics and their implications for tissue engineering in reproductive medicine. It emphasizes the importance of developing engineered tissues that closely resemble their natural counterparts, and their use in advancing reproductive medicine and regenerative therapies.

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Abstract theme: Biomaterials

CHEMICALLY CROSSLINKED PROTEIN HYDROGELS WITH GENETICALLY ENCODED BIOACTIVE DOMAINS AS CUSTOMISED MATRICES FOR 3D CELL CULTURE

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Introduction

Hydrogels are very important for tissue engineering. They can act as a matrix to support mammalian cell growth thanks to their unique characteristics, namely, tissue-like water content and mechanical properties. Typically, hydrogels are made of either synthetic polymers or natural source proteins/polysaccharides. The former show good mechanical properties but poor biological activity, whereas the latter offer better biological activity but poorer mechanical properties and high batch-to-batch variability, a common issue with the widely used Matrigel. A novel approach to overcome this trade-off is to make hydrogels using rationally designed proteins incorporating genetically encoded bioactive domains.

The Regan lab has developed such recombinant protein hydrogels using SpyCatcher-SpyTag[1] chemistry. The protein network is made of two building blocks, the first is a tandem array of up to six SpyCatcher domains and the second is a rod-like repeat protein derived from *S.aureus* surface protein G (SasG), with a SpyTag peptide fused to it at each end. Upon mixing, they spontaneously react with each other, creating a covalently crosslinked hydrogel network. SpyCatcher arrays have been designed to incorporate such bioactive domains as an RGD integrin binding site for improved cell adhesion and a matrix metalloproteinase (MMP) sensitive cleavage site for tunable hydrogel degradation.

Materials and Methods

SpyCatcher arrays have been cloned using a modular approach, in which a gene encoding for three SpyCatcher domains was PCR amplified with primers containing bioactive domains in their overhangs, and then Gibson assembly was used to assemble the plasmid. All proteins were expressed in *E.coli*, purified using Ni-NTA affinity chromatography and lyophilised for storage. SDS-PAGE was used to confirm that the size of the purified protein matched the expected size.

SpyCatcher arrays and SpyTag-SasG-SpyTag crosslinkers were re-suspended in DMEM at pre-determined concentrations, and then mixed to prepare hydrogels. Hydrogels' mechanical properties were characterised using dynamic shear rheometry. Their biological properties will be tested with HepG2 cells to establish the impact of the different bioactive domains on cell behaviour and proliferation.

Results and Discussion

Four different versions of SpyCatcher arrays have been successfully designed, cloned, expressed and purified. The first one is a 'standard', with six SpyCatcher domains only (SC6); the second one contains an additional RGD site for better cell adhesion (SC6 RGD); the third one contains an additional matrix metalloproteinase (MMP) cleavage site for better hydrogel network degradation (SC6 MMP), and the last one has both the RGD and MMP cleavage sites (SC6 RGD MMP).

We have shown that upon mixing SpyCatcher arrays with SpyTag-SasG-SpyTag crosslinkers, hydrogels form within 5 to 30 minutes, depending on the concentration of the precursor solutions. Regarding mechanical properties, amplitude sweeps revealed that hydrogels did not yield until approximately 100% shear strain, showing ductile behaviour. Frequency sweep revealed that hydrogels' shear storage modulus in the equilibrium region was 180 Pa, which indicates a rather soft material, but is similar to other protein-based hydrogels reported in the literature[2].

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ENGINEERING A BIOMIMETIC 3D BREAST TUMOUR MODEL FOR THERAPEUTIC SCREENING

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Introduction Breast cancer is the most common malignancy affecting women worldwide. The breast tumour microenvironment comprises specific biophysical, biochemical and cellular facets, which include a collagen rich extra-cellular matrix and a mixture of tumour cells and stromal cells such as mesenchymal stem cells, immune cells, fibroblasts, adipocytes and vascular cells. Current research demonstrates the importance of crosstalk between the different cell types located within the tumour microenvironment (TME) in breast cancer progression and therapy resistance [1]. Three-dimensional (3D) biomimetic cell culture models have been developed to study breast cancer due to their capability to better mimic the physiological 3D tissue architecture of the tumour microenvironment. Engineering 3D tumour models of breast cancer have evolved from simplistic suspension-triggered spheroids, synthetic scaffolds, natural and synthetic hydrogels, and more recently emerging sophisticated bioprinted matrix models [2]. Tumouroids are spatially compartmentalized 3D collagen I models that mimic the tumour microenvironment by embedding a cancer mass within a stromal compartment. They exhibit physiologically relevant cell-cell and cell-matrix interactions, gene expression and signaling pathway profiles, heterogeneity and structural complexity that reflect *in vivo* tumours [3]. The present study aims to develop a biomimetic 3D culture breast tumour model for mechanistic studies and screening of drugs.

Materials and Methods Multi-compartment dense collagen I gels were engineered, where either MCF-7 or MDA-MB-231 breast cancer cells were embedded within a central artificial cancer mass, and in the surrounding stromal compartment, adipose tissue derived mesenchymal stem cells were seeded in addition to endothelial cells with 20 µg/ml laminin and 2 mg/ml fibrin. The multi-compartment gels were cultured for 21 days then stained with CD31 and pan-cytokeratin antibodies followed by fluorescence imaging. Images were examined for the formation of endothelial networks and cancer invasion. VEGF levels were measured along with mapping of oxygen gradient levels in 3D models. Free doxorubicin and a liposomal formulation of doxorubicin at 10 µM each were applied for 48 hours for therapeutic efficacy testing through measurement of cell viability, VEGF levels, oxygen gradient levels.

Results and Discussion Presence of adipose-tissue derived mesenchymal stromal cells and extracellular matrix proteins (20 µg/ml laminin and 2 mg/ml fibrin) in the stroma supported vascular network formation. Tumouroids containing MCF-7 breast cancer cells developed more complex vascular network compared to MDA-MB-231 ($P \leq 0.01$). Vascularized stroma of MCF-7 3D tumouroids promoted breast cancer invasion ($P \leq 0.05$) as opposed to MDA-MB-231. Real-time monitoring of oxygen gradients in 3D tumouroids demonstrated that MDA-MB-231 breast cancer cells were more hypoxic ($P \leq 0.05$) than MCF-7 cells which also correlated to higher VEGF levels ($P \leq 0.001$). Stromal composition significantly ($P \leq 0.0001$) influenced hypoxia development in MCF-7 breast cancer mass as opposed to MDA-MB-231. Doxorubicin was found to be more effective than its liposomal formulation in reducing cell viability in both MCF-7 and MDA-MB-231 tumouroids correlating with reduced VEGF levels and increased oxygen saturation levels. Moreover, it was noted that doxorubicin killed more MDA-MB-231 breast cancer cells than stromal cells in contrast to MCF-7.

Conclusions This work demonstrates the development of biomimetic 3D breast tumour model and the influence of complex stroma on hypoxia development, breast cancer invasion and therapeutic response to various drugs.

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MIMICKING THE LEUKEMIC MICROENVIRONMENT VIA USING SOFT POLYETHYLGLYCOL GELS

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Abstract theme: Bioengineered Models / Mechanobiology

Introduction: Leukemia is a group of hematopoietic malignancies that can be mainly classified to myeloid or lymphocytic according to the cell from which the disease was originated while acute or chronic depending on the stage of the disease (1). Acute myeloid leukemia is the most lethal and most prevalent among all the leukemic types while patients that are relapsed or refractory for standard chemotherapy can only benefit from hematopoietic stem cells transplantation (HSCT). However, even then, up to 55% of the patients will experience disease relapse while after this their prognosis is poor (2, 3). HSCs are the regulators of hematopoiesis nevertheless, mutations can lead to their differentiation arrest, malignant expansion, and leukemogenesis (4). All the currently available *in vivo* models fail to recapitulate the tumor microenvironment comprehensively (5); therefore, the development of humanized models might confer a greater potential. **Materials & Methods:** In this project we culture bone marrow hMSCs in aggrewell plates as spheroids, followed by their encapsulation in soft laminin-biofunctionalized polyethylglycol gels. THP1 (leukemic cells) are added outside of the insert which contains the gel, allowing the remodeling of the niche via their secretory profile. The leukemic niche is treated with daunorubicin chemotherapy to investigate its effects on both the THP1s and spheroids. Assessment of viability of the spheroids was performed via live/dead staining while the viability of leukemic cells was assessed via Alamar blue, and MTS. ELISA was used to characterize the secretory profile of the spheroids after the addition of the THP1s. Immunofluorescence was used to investigate the protein expression as well as morphology of spheroids. **Results:** Preliminary data have shown that the addition of THP1s does not affect the viability of the spheroids. However, the THP1s induce the disaggregation and deformation of spheroids. The spheroid activation results in disaggregation, which allows single cells to escape as well as more elongated spheroids which tend to secrete more ECM proteins such as fibronectin and collagen. Furthermore, the addition of THP1s, upregulates the secretion of IL-6, the most prominent marker of chemoresistance in the leukemic niche. The addition of chemotherapy does not affect the viability of the spheroids due to their reduced proliferation and perhaps because of their quiescent phenotype. Nevertheless, it dramatically affects the viability of the THP1s. The presence of spheroids contributes to the development of chemoresistance in the niche, potentially via the secretion of IL-6. Finally, it has been observed that the addition of leukemic cells educates the MSC spheroids towards an adipogenic phenotype as well as to the upregulation of stemness associated markers such as the nestin. Moreover, a downregulation on the tumour suppressor p53 can be observed potentially because of the spheroid activation. **Discussion:** Overall, we believe that the development of humanized models can mimic more precisely the tumour microenvironment at different stages of the disease. Moreover, the development of a model that accurately recapitulates leukemogenesis as well as leukemic progression can facilitate the reliable screening of novel anticancer therapies. More importantly, we propose that the current model has the potential for development of personalized models that could be used for the testing of targeted therapies. Future experiments will focus on the addition of HSCs in the leukemic niche followed by assessment of their phenotype. **References:** 1. Hao T, *et al.* Sci Rep. 2019;9(1):12070. 2. Shallis RM, *et al.* Blood Rev. 2019;36:70-87. 3. Bose P, *et al.* Leukemia. Curr Treat Options Oncol. 2017;18(3):17. 4. Akinduro O, *et al.* Nat Commun. 2018;9(1):519. 5. Almosailleakh M , *et al.* Int J Mol Sci. 2019;20(2). **Acknowledgements:** We would like to specially thank all the supervisors for their guidance, our industrial partner Cell Guidance Systems, the ECMage Network as well as all the CeMi members for their support. Also, we would like to thank MRC for funding this project.

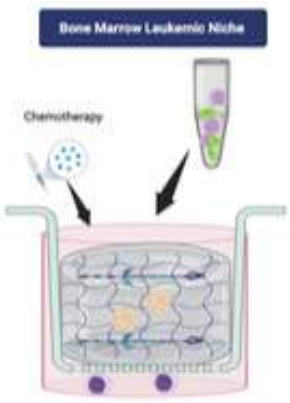

Figure 1. *Schematic representation of the leukemic model.*

UNCOVERING THE SECRETED SECRETS OF ADIPOSE TISSUES TO ENGINEER NOVEL CELL-FREE THERAPIES FOR SCAR TISSUE REGENERATION

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Introduction

Scar formation is a crucial step in wound healing that is in part driven by myofibroblasts. Prolonged myofibroblast activity results in excessive collagen deposition and adverse scar formation including hypertrophic or keloid scars. Factors produced from adipose tissue and adipose-derived stromal cells (ADSCs) have demonstrated regenerative properties that are beneficial for wound healing [1]. The aim of this project is to determine the mechanism through which secreted factors from adipose tissue are able to support scar tissue regeneration.

Materials and Methods

Conditioned media was generated by incubating ADSCs or adipose tissue for 72 hrs in serum free media. The regenerative effects of conditioned media were tested using a simple *in vitro* scar model. The model consisted of human dermal fibroblasts cultured with transforming growth factor beta (TGF-β) and conditioned media from ADSC or adipose tissue.
Conditioned media was characterised and the expression of factors associated with myofibroblast differentiation were measured using qualitative and quantitative techniques including: qPCR, ELISA, and immunocytochemistry.

Results and Discussion

Using immunocytochemistry, we found that treatment with adipose tissue conditioned media reduced the proportion of smooth muscle actin positive cells following stimulation with TGF-β compared to controls, but the same effect was not observed following treatment with ADSC conditioned media. This is consistent with qPCR data, which showed a decrease in the gene expression of factors associated with myofibroblast differentiation (alpha smooth muscle actin, collagen 1 and fibronectin) following treatment with adipose tissue conditioned media .

When examined by ELISA, adipose tissue conditioned media contained significantly higher levels ($p<0.01$) of the pro-inflammatory cytokines IL-6 and IL-8 compared to ADSC conditioned media.

Conclusions

In conclusion, secreted factors found in adipose tissue conditioned media were able to reduce the expression of factors related to scar tissue formation and myofibroblast differentiation, in particular, the expression of smooth muscle actin. Further work is now required to determine which factors are responsible for these effects and how this could be utilised to support scar tissue regeneration.

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THE EFFECTS OF PORE SIZE AND GEOMETRY ON THE PERFORMANCE OF BONE TISSUE SCAFFOLDS

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Introduction

Despite the natural ability of bones to regenerate, severe loss of bone tissue due to degenerative conditions, tumour resection, or trauma requires reconstructive intervention [1]. Bone Tissue Engineering (BTE) aims to replace injured tissue with three-dimensional bio-active scaffolds [2]. Aligned with the goals of bone tissue engineering, this research has designed, developed, and tested forty novel scaffold designs composed of Polylactic Acid in order to investigate their efficiency as bone substitutes.

Materials and Methods

A series of design parameters such as scaffold shape, pore size, porosity, and angle of orientation were used to achieve sophisticated functional scaffolds with various combinations of geometrical structures. Due to numerous inherent challenges of tissue engineering experiments, Finite Element Analysis (FEA) has been utilised to predict the mechanical performance of the scaffolds under mechanical loading. The boundary conditions of the FEA were defined in accordance with the natural load-bearing conditions of femur bones. The designs were fabricated using an FDM printer and PLA filament, and mechanical tests were performed to validate the reliability of the simulations.

Results and Discussion

Stress analysis indicated that eight out of forty proposed designs fulfil the required mechanical strength to be used as bone scaffolds with their equivalent stress lying within the acceptable limits of yield compressive strength for human cortical bones. The porosity of the eight scaffolds ranged from 39.89 to 57.88%. Previous studies have suggested that this range is optimal for growth and differentiation of osteoblastic cells [3]. Moreover, due to having several angular arrangements between the respective layers of the geometries, the scaffolds possess both micro and macro pores. This combination of pore sizes exhibits superior resemblance to the natural microenvironment of human bones compared to conventional scaffolds with uniform pore structures. Additionally, it accommodates both fluid and nutrient transport and provides optimal spaces for osteocyte activity [4]. Compression tests closely resembled the computational data with an average variance of 4.2%, confirming the accuracy of FEA studies.

Conclusion

This research investigated the effect of pore size, pore geometry, and porosity on the performance of bone scaffolds. It was concluded that eight out of forty proposed geometries meet the requirements for both mechanical and biological activity. Experimental tests focused on mechanical compression were in agreement with computational data obtained from FEA. Future research direction involves biological studies to evaluate the bioactivity of the designs.

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Abstract theme: Biomaterials

ADIPOSE DERIVED DECELLULARIZED EXTRACELLULAR MATRIX FOR SOFT TISSUE APPLICATIONS

Introduction

Decellularized extracellular matrix (dECM) derived from adipose tissue offers a biocompatible scaffold that closely emulates the native extracellular environment, fostering favorable conditions for cell adhesion, proliferation, and differentiation. This inherent biocompatibility is pivotal for facilitating the seamless integration of vascular cells and the formation of intricate vascular networks within 3D-printed constructs. There is increasing interest in the development of printable inks based on dECM, and various strategies are being progressed, such as methacrylation. This work explores the derivatization of dECM from adipose tissue by carbic anhydride to result in dECM-norborene (dECM-Nb). This can be used in ink formulations in combination with gelatin-norbornene (Gel-Nb) and di-thiols, exploiting thiolene click reactions, and offering customizable properties suitable for various 3D printing applications and biological functionality. Leveraging its natural composition and architecture, dECM from porcine adipose tissue serves as an ideal template for orchestrating vascular network formation, enabling the infiltration and organization of endothelial cells and pericytes. Moreover, the dECM retains vital bioactive molecules, including growth factors and signaling proteins, which orchestrate crucial processes such as angiogenesis and vascular development.

Materials and Methods

Fresh porcine adipose tissue was harvested, cleaned, and cut into small pieces (~2-3 mm^3) before being immersed in a detergent solution to lyse cells and solubilize cellular components. After rinsing with sterile PBS, the tissue was treated with an enzyme solution supplemented with protease inhibitors to digest residual cellular material. Following digestion and further rinsing, the decellularized ECM (dECM) was then freeze-dried. The dECM was dissolved in weak acetic acid solution and derivatized using carbic anhydride at room temperatures for 3h to result in dECM-Nb. Derivatization was assessed by ^{1}H-NMR analysis. Gelatin-norbornene (Gel-Nb) was prepared by reaction of porcine gelatin with carbic anhydride at a temperature of 40 °C for 48h, degree of substitution was confirmed by ^{1}H-NMR, as well as o-phthalaldehyde (OPA) assay.

Results and Discussion

The decellularization protocol removed ~95.0% of the DNA content from the adipose tissue. Adipose dECM was successfully derivatized with norbornene groups, as shown in Figure 1. The degree of substitution of Gel-Nb was determined using three corroborative techniques to be 71.4% by ^{1}H-NMR, 72.7% by TNBS assay, and 84.6% by OPA. The dECM-Nb and Gel-Nb system can be combined to make tailorable hydrogels using dithiothreitol (at 0.05 wt.%) and the photoinitiator lithium phenyl (2,4,6-trimethylbenzoyl) phosphinate (LAP) at 0.05 wt.%.

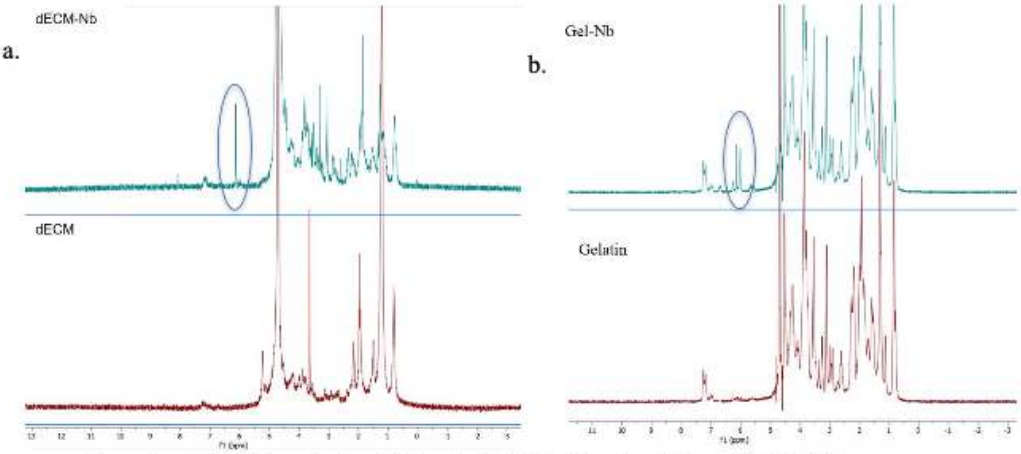

Figure 1 ^{1}H-NMR results for dECM and dECM-Nb (a) and gelatin and Gel-Nb (b)

Conclusions

Adipose derived dECM-Nb has been produced and characterized. This bioink component holds significant promise for soft tissue engineering applications and can be combined with Gel-Nb to make tailorable hydrogels. It is a challenge to produce significant quantities of dECM-Nb, but by combining this with Gel-Nb as an additive allows for production of hydrogels with enhanced bioactive cues.

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PERFORMANCE ASSESSMENT OF 3D-PRINTED PLA SCAFFOLDS FOR WOUND HEALING AND ANTIBACTERIAL ACTIVITY

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Abstract theme: Biomaterials

Introduction: Tissue engineering plays a crucial role in promoting overall health by facilitating the repair and regeneration of damaged tissues. However, bacterial resistance to antibiotics poses a growing concern, urging the exploration of novel antibacterial compounds derived from organic or inorganic materials. *Bienertia sinuspersici*, a species discovered in the Arabian Gulf region, is believed to have potential antibacterial activity owing to its adaptation to extreme environmental conditions.

Materials and Methods: In this study, we investigate the efficacy of various polylactic acid (PLA) scaffold designs in supporting cell adherence and growth, particularly with Human Skin Fibroblasts (HSFs) and reduce bacterial growth such as *Staphylococcus epidermis*, with and without coating with *Bienertia sinuspersici* leaves extraction. The scaffolds were fabricated using a 3D printing technique, with porosities ranging from 20% to 100%.

Results: The preliminary results (without coating) revealed that scaffold designs with porosities of 60%, 80%, and 100% exhibited promising adherence of HSFs, with the 80% and 100% designs being notably effective. Imaging techniques, including Scanning Electron Microscopy (SEM), were employed to visually assess cell adherence on the scaffolds. The 60% scaffold design demonstrated good cell adherence, as confirmed by cell viability tests using the AlamarBlue assay, suggesting enhanced cell viability compared to the 20% design. Additionally, bacterial culture experiments showed variations in bacterial adherence across scaffold designs, with the 20% porosity scaffold exhibiting a higher bacterial population, as indicated by optical density measurements.

Conclusions: Overall, tthis study may provides valuable insights into scaffold design considerations for tissue engineering applications, shedding light on potential strategies to enhance cell adherence and combat bacterial contamination in wound healing application.

A PHOTOCROSSLINKABLE INJECTABLE HYDROGEL SYSTEM TO FACILITATE THE REPAIR OF CARTILAGE LESIONS

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Introduction: Osteoarthritis, a degenerative joint disease, arises from cartilage breakdown due to aging, stress, or injury. Current therapies, including matrix-induced autologous chondrocyte implantation (MACI), are associated with invasiveness and high costs, often failing to achieve optimal hyaline cartilage regeneration. Injectable hydrogels have emerged as a promising alternative, mimicking native cartilage properties to promote tissue repair. However, existing injectable hydrogels exhibit inadequate adhesion to the defect site, particularly in the presence of fluid during arthroscopic surgery. Overcoming these challenges is imperative for the development of effective and minimally invasive treatments for osteoarthritis.

Materials and Methods: We developed an adaptable injectable hydrogel system tailored for underwater cartilage repair. Initially, an aldehyde-functionalized hyaluronic acid methacryloyl (Ald-HAMA) hydrogel was applied as a coating onto the cartilage defect, serving as a priming layer. This layer established a robust and permanent connection with the defect site through covalent Schiff-Base linkages. In the subsequent step, a hydrogel consisting of gelatin methacryloyl (GelMA), HA methacryloyl (HAMA), and methacrylated platelet lysate (PLMA) was introduced on top of the primary layer. A 60 sec. exposure to UV-light (λ=365nm) initiated photopolymerization, ensuring the covalent bonding between the two hydrogel layers. Assessment of hydrogel cytocompatibility was performed using LIVE/DEAD staining, while dynamic changes within hydrogel constructs were monitored by evaluating glycosaminoglycan (GAG) and DNA content over time. An in-depth histological and immunohistochemical analysis was conducted on the hydrogel using an ex-vivo cartilage defect model to assess its chondrogenic potential. Mechanical testing and rheological analysis were employed to assess the injectability and structural stability of various hydrogel formulations under aqueous conditions.

Results and Discussion: All hydrogel formulations displayed excellent injectability, enabling minimally invasive fluid-filled arthroscopy, with gelation occurring in less than 60-sec, as confirmed by rheological testing. Ald-HAMA exhibited higher adhesive strength compared to GelMA/HAMA, surpassing even clinical tissue sealants. Cell-laden constructs exhibited impressive viability of ~90%, with PL integration concurrently promoting GAG secretion. Protein release assays demonstrated sustained release of essential growth factors when PL was covalently bonded to the hydrogel, further facilitating effective cartilage repair. Immunofluorescence analysis revealed increased type-II collagen content resembling that of hyaline cartilage.

Conclusions: This study introduces a novel injectable hydrogel system for underwater cartilage repair. Our two-step approach demonstrates exceptional injectability, adjustable mechanical stiffness, and strong adhesive properties. Furthermore, the hydrogel promotes cell viability and chondrogenic activity, showing promising potential for hyaline cartilage repair.

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BIOENGINEERING SURFACES TO PRESERVE MESENCHYMAL STROMAL CELL GROWTH *IN VITRO*

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Introduction

Mesenchymal stromal cells (MSCs) have been identified as a promising candidate for use in cell therapies due to their unique properties such as multipotency (tissue regeneration) and immune-modulation (reducing transplant rejection)[1]. MSCs have the ability to differentiate into various cell types such as osteoblasts, adipocytes, chondrocytes, and fibroblasts [1,2]. However, in order to use them for cellular therapy, many millions of cells from a single donor are required for allogeneic approaches [3]. Further, maintaining their immunomodulatory and multipotent phenotype is of critical importance during cell expansion. Unfortunately, while growing these cells in cell culture, a common challenge is encountered. MSCs easily differentiate into other cell types (phenotypical drift) or growth exhaustion *in vitro* [3]. This is because the cell culture differs significantly from the natural environment of the MSCs, their niche. It is widely known that MSCs are greatly influenced by their environment [4]. In the bone marrow (BM) niche, MSCs have intermediate levels of intracellular tension, while osteoblasts have higher tension levels and adipocytes have lower tension levels [4]. In light of this understanding, we hypothesise that the growth of MSCs can be supported *in vitro* by modifying the cell culture environment, and in turn, obtain a higher yield of high-quality MSCs. Here, we use laminin, an extracellular matrix protein that has been shown to promote cell adhesion while lowering intracellular tension, organised by polyethylacrylate (PEA) [5,6,7]. PEA is a polymer that has been shown to organise extracellular matrix proteins in a more biomimetic manner [8,9].

Materials and methods

To optimise cell culture conditions, the culture surfaces are coated with PEA. PEA was applied by plasma polymerisation of the monomer, ethylacrylate, onto polystyrene culture plates. Stro-1 selected MSCs were cultured on the surfaces for a certain time depending on the technique. Following culture, qPCR transcript analysis was used to quantify p16, p21 and p53, and In-Cell Western protein analysis was used to quantify caspase3, caspase6 and Retinoblastoma protein (RB), extracellular signal-regulated kinase (ERK), and cyclin- dependent kinase (CDK). Further, immunofluorescent microscopy was used to study nuclear abnormalities and nuclear lamina components, especially Lamin A/C.

Results and Conclusion

According to the results obtained from the different techniques used, MSCs exhibited better growth on PEA- lamin compared to on tissue culture plastic. They expressed less age-related markers and exhibited lower levels of lamin A/C, a marker of MSC phenotype. The data provides evidence that better conditions, that draw on the natural cell interactions with their extracellular matrix, can be used to produce better cells for cell therapies. The mechanism behind the process remains to be investigated.

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DEVELOPMENT OF DERMAL MATRIX WITH REVASCULARIZATION PROPERTIES TO ADVANCE THE INTEGRATION OF SKIN SUBSTITUTES.

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Introduction: The treatment of large skin defects due to burns or trauma remains a major healthcare challenge. Nowadays, skin grafts and dermal substitutes are still the standard practice for major skin defects. Despite progress in artificial dermal grafts and the notion that thin tissues rely on oxygen diffusion from surrounding vessels and ingrowth of vessel-forming angiogenic cells has shifted attention away from the significance of revascularization in dermal graft regeneration. However, the success of a skin graft or "take" is highly dependent on the revascularization of the graft. Revascularization of the skin is essential for nutrients, oxygen, and immune cell supply in the healing of wounds [1], [2]. This project aims to develop acellular dermal substitutes that are capable of inducing angiogenesis that will better support their integration for the treatment of large skin defects arising from burns or trauma.

Materials and Methods: Three different peptides with angiogenic properties were assessed *in vitro* for their effectiveness on endothelial cell chemotaxis and proliferation. HUVECs (Promocell, UK) were cultured in EGM-2 endothelial cell medium (Lonza, UK) and prior to each assay were deprived of growth factors for 4h. To demonstrate different aspects of angiogenesis wound closure assay utilising the Ibidi insert (Ibidi GmbH, UK), 8µm pore transwell (Greiner Bio-one, UK) cell migration and collagen type I (Merck Corning, UK) invasion assays were performed in the presence of each peptide. At least 10 random field images were captured with the EVOS (Thermo Fisher, US) optical microscope or the CQ1 (Yokogawa, Japan) confocal fluorescent microscope for fluorescently labelles cells.

Results & Discussion: The candidate peptides demonstrate different capacities to enhance proliferation, migration, and invasion. The wound closure assay and migration assay demonstrated that the presence of the peptides in comparison to deprived growth factor cell culture media induce cell migration and proliferation. The cell invasion assay utilizing a transwell coated with collagen I matrix demonstrated that the chemoattractant effect of the peptides enhanced HUVECs extracellular matrix (ECM) invasion. Derived from different angiogenic growth factors and molecules, the peptides activate different pathways and enhance different aspects of angiogenesis. Insights into the development of a new angiogenesis in vitro model will play a pivotal part in the development of this project. The most successful peptide candidate will be chemically incorporated into bioactive polymers to develop 3D-printed bioactive dermal matrices.

Conclusion: Our results show that characterization of the angiogenic peptides according to its potential to direct HUVEC proliferation, ECM invasion and migration allowed for screening of the successful candidate to be incorporated in bioactive angiogenic dermal graft materials. Enhancing a rapid migration of endothelial cells and proliferation is necessary to provide enough to demonstrate adequate new vessel ingression. However, inducing better stability and functional organization of the network are also primordial to the growth of a perennial network.

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HYDROGEL ENCAPSULATED BONE MARROW STEM CELL DERIVED EXTRACELLULAR VESICLES FOR BONE REGENERATION

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Introduction: Bone tissue is the second most transplanted tissue in the body, worldwide, with over 4 million bone transplantations occurring, annually [1,2]. Bone marrow-derived stem cells (BMSCs) have been extensively used in bone tissue engineering (BTE) as components for bone constructs due to their osteoblastic lineage commitment and their ability to indirectly promote tissue repair through the paracrine trophic effects they secrete [3]. Evidence supports the beneficial effects of BMSCs are due to their paracrine action mediated via the release of the extracellular vesicles (EVs) [4,2]. BTE has made a great progress in bone regeneration by developing 'bone substitutes' using bioactive materials. In this project, in collaboration with Sphere Fluidics Limited [5], we are aiming to manufacture a novel BMSC-EV microenvironment using GelMA hydrogel beads where osteogenic primed EVs will be encapsulated within the beads and used as a cell-free therapy mechanisms for bone regeneration and repair.

Materials and Methods: MSCs were osteogenically primed for 28 days using two distinct methods: (i) chemical induction, using osteogenic media containing dexamethasone and the bone morphogenic protein 2 (BMP2) growth factor, and (ii) mechanical induction, through nanoscale vibrational displacements, termed nanokicking [6]. Verification of BMSCs osteogenic differentiation was characterised at a protein and histological level, using western blot assay and alizarin red staining, respectively. BMSC-derived EVs were isolated (differential centrifugation, ultrafiltration, and size exclusion chromatography) and extensively characterised in terms of size and concentration (microBCA, dynamic light scattering, tunable resistive pulse sensing, nanoparticle tracking analysis, nano flowcytometry, and transmission electron microscopy). Subsequently, both 5% and 7.5% GelMa hydrogels were prepared and their mechanical properties where characterised (rheology). Naïve BMSCs and CFSE fluorescently labelled EVs are to be encapsulated within the hydrogels and imaged under confocal microscope to ensure uniform EV distribution throughout the hydrogel.

Results and Discussion: Both the protein analysis and the histological assay confirmed BMSC osteogenesis via chemical and mechanical induction when compared to the non-treated BMSCs (negative control). Successful EV isolation was verified using the techniques described. EVs appeared to be in the same size range (100-250nm) when isolated under both chemical and mechanical induction as well as the negative control, however EV concentration was slightly increased in the chemically treated BMSCs, perhaps indicating cell stress. Both the 5% and 7.5% hydrogels shared elastic properties, with the 7.5% ones having a higher stiffness, compared to the 5% ones, a property essential for osteogenesis. CFSE stained EVs are expected to be evenly distributed withing the hydrogels.

Conclusion: BMSC analysis indicated osteogenesis occurring during both chemical and mechanical stimulation, with the latter being a slightly slower process. BMSC-EVs will be characterised in terms of their cytokine cargo in both chemically and mechanically induced populations and will be compared to the BMSC-EVs derived from the negative control BMSCs. Osteogenically induced EVs are expected to bare cytokimes stimulating osteogenesis in naïve BMSCs as opposed to the negative control EVs. CFSE stained BMSC-EVs are expected to be more evenly distributed across the 7.5% hydrogels in comparison to the 5% hydrogels. In collaboration with Sphere Fluidics Limited, a novel microfluidic EV environment composed by 7.5% GelMA hydrogel beads encapsulating MSC-EVs and naïve stem cells, will be manufactured, to evaluate the ability of the osteogenic derived BMSC-EVs to promote osteogenesis in naïve BMSCs for bone regeneration and repair.

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Bioengineered Models

THE RE-CREATION OF THE INTESTINAL EPITHELIUM USING INDUCED PLURIPOTENT STEM CELL DERIVED PROGENITORS AND 3D BIOPRINTING FOR REGENERATIVE MEDICINE APPLICATIONS

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Introduction: Understanding the anatomy and physiology of intestine epithelium is significant for regenerative medicine applications and disease modelling because of the complex intestinal epithelium architecture and cell signalling that determines stem cell fate [2]. Induced pluripotent stem cells (iPSCs) are self-renewing cells that can differentiate into different lineages, including ectoderm, mesoderm, and endoderm [3]. We have recently developed a new protocol for the derivation of intestinal progenitor cell populations from human iPSCs [4]. The emergence of 3D bioprinting techniques alongside the advent of iPSC technologies opens the door to new opportunities in regenerative medicine for intestinal conditions and to recreate the complex architecture of the intestinal epithelium [5]. This study aims to recreate the geometry of the intestinal epithelial stem cell niche and the underlying stroma through 3D bioprinting.

Materials and Methods: Gelatin Methacrylate (Gel-MA) was synthesised via the precipitation method and chemically characterised via FTIR and ^{1}H-NMR to evaluate the presence of methacryloyl groups. Gel-MA inks were prepared by dissolving 10, 20, and 30% (w/v) Gel-MA, 1% (w/v) photoinitiator (LAP), and 0.025% (w/v) photoabsorber (Tartrazine) in PBS at 50 °C. Gel-MA printability was confirmed by fabricating 8 mm diameter discs from different Gel-MA concentrations. Caco-2 cell viability on Gel-MA discs was evaluated via a metabolic activity assay (Presto Blue) and Live-Dead staining. Mesenchymal stromal and epithelial intestinal cell populations were differentiated from iPSCs through a robust biochemical protocol as confirmed via qPCR and flow cytometry [4]. To ensure biocompatibility of each stage of the Gel-MA printing process, different UV exposure durations (15s, 30s, 45s, and 60s) were presented to cells at seeding and viability in subsequent culture assessed over the following 7 days. Additionally, iPSC-derived intestinal mesenchymal stromal cells (IMSC) were temporarily incubated in the 10% Gel-MA ink before removal and plating to evaluate any cytotoxic effect of the precursor Gel-MA bioink on cells over the following 7 days.

Result and Discussion: FTIR confirmed the reaction between Gelatin and methacrylate anhydride occurred through analysis of the C=C bonds. The degree of methacrylation of gelatin was calculated using ^{1}H NMR spectra and found to be 65%. Gel-MA-based discs were non-toxic according to cytotoxicity assay (Presto Blue) and Live-Dead staining. qPCR analysis showed differentiated iPSCs expressed *CDX2, SHH* (genes expressed in normal colonic epithelial cells), and *SOX6, ACTA2, COL1A1* (genes expressed in normal colonic mesenchymal cells), illustrating iPSCs successfully differentiated into intestinal epithelial progenitor and mesenchymal stromal cells. Flow cytometry demonstrated that 99.8% of live epithelial cells were EPCAM+.

UV-exposed iPSC-derived IMSCs showed a dose-dependent decrease in cell viability in comparison to control cells (non-exposed) for the first three days. However, by day 7 of culture, viability assays revealed recovery of the exposed groups. iPSC-derived IMSCs temporarily exposed to Gel-MA Ink showed a cell viability exceeding 86% of the control following 7 days of culture, classifying it as non-cytotoxic (>70% is the threshold for biocompatibility ISO 10993-5 [6]).

Conclusion: This study demonstrated that 3D-printed Gel-MA hydrogels are biocompatible with Caco-2 cells. Furthermore, any reduction in cell number or viability due to incubation with Gel-MA bioink and/or UV exposure times of up to 60s is recovered within 7 days, demonstrating the biocompatibility of the printing process for iPSC-derived intestinal cells. Future studies will directly print these cell types within the Gel-MA to recapitulate the intestinal epithelium and provide a supportive architecture to recreate the native intestinal mucosa.

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TUNEABLE MICROGELS FOR GUIDING CELLULAR RESPONSE IN TISSUE REPAIR

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Abstract theme: Bioengineered Models / Biomaterials

Introduction: Controlled delivery of therapeutic proteins, such as growth factors, is a highly promising treatment strategy in wound healing and deteriorative conditions, such as non-union bone defects [1]. The primary obstacle in successful protein therapy lies in the lack of injectable carriers that are both biocompatible and capable of delivering proteins directly to the affected area with optimal bioactivity and controlled release rates. Hydrogels, highly hydrated 3D cross-linked polymer networks, have been studied as potential protein carriers that can mimic the properties of the extracellular matrix in native tissues. The encapsulation of bioactive molecules within the hydrogels would allow for the targeted and sustained delivery to the site of defect or injury, providing enough time for the tissue to heal and stimulating the tissue growth and cell differentiation. Injectable microgels with highly controlled biomechanical properties and protein release mechanisms can be developed, allowing a minimally invasive administration in the site of injury [2]. Microgels can be produced by using microfluidics approaches, that allow the high-throughput production of size-controlled, spherical and monodisperse microparticles [2]. Natural collagen- and synthetic poly(ethylene) glycol (PEG)-based scaffolds are promising biomaterial candidates for the microgel production for the applications such as bone and cartilage tissue repair [3]. The aim of this project is the development of controllable and robust natural and synthetic microgel platforms for the sustained protein delivery and modulation of cellular response by designing collagen-based (COL) and maleimide-functionalised (PEG-MAL) microgels, via droplet-based flow-focussing microfluidics, with tuneable mechanical properties and degradation rates.
Materials and Methods: Microfluidic devices with flow-focussing geometry and 200 µm nozzle size were produced by casting PDMS onto a silicon-based wafer. COL microgels are produced by crosslinking collagen with 8arm-PEG via NHS-ester chemistry in a 2:1 ratio using fluorinated oil as a continuous phase. PEG-MAL microgels are synthesised by crosslinking PEG-MAL macromers with PEG-dithiol via a Michael-type addition chemistry in a 1:1 ratio, at a buffer pH of 5.6, using mineral oil as a continuous phase. The polymer and crosslinker solutions are pumped inside the microfluidic device using syringe pumps through designated inlet channels and collected from a serpentine-like outlet channel. The droplets are then washed from the oil suspension and analysed for the size distribution and mechanical properties via nanoindentation. Protein encapsulation is carried out by incorporating the labelled protein in the crosslinking phase, and the release is evaluated by analysing the supernatant in a plate reader. Different strategies are investigated with the aim of increasing the sustained release of the encapsulated protein. Cell viability on the microgels is assessed on the mesenchymal stem cells (MSC) using a LIVE/DEAD assay, and cell adhesion is evaluated via imaging.
Results and Discussion: COL and PEG-MAL microgels were successfully synthesised inside a microfluidic device with a diameter of 200±20 µm and 100±20 µm, respectively, and FITC-BSA was successfully encapsulated inside the microgels and bulk hydrogels. The protein release was evaluated at different time points for 5 days for the COL system. The protein encapsulation in microgels entrapped in a bulk hydrogel provided a granular network that improved the sustained release compared to the direct encapsulation in the bulk hydrogel. MSC viability and proliferation on the COL bulk 2.5D system was performed in preparation for the *in vitro* studies involving the COL microgel system, followed by the assessment of viability on microgels.
Conclusions: We are developing a tuneable microgel platform for the encapsulation of proteins and guiding cellular response, involving collagen- and PEG-based scaffolds, via microfluidics, where the protein release and MSC behaviour can be precisely controlled by fine-tuning the microgel composition and size.

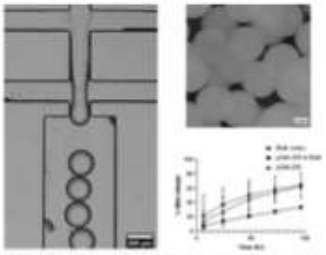

Fig 1. FITC-BSA protein encapsulation and release in the COL microgel system.

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Acknowledgements
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Abstract theme: Biomaterials

Title: **BILAYER SCAFFOLD COMBINING ELECTROSPUN PCL AND A POROUS GEL LAYER FOR ENHANCED GUIDED BONE REGENERATION**

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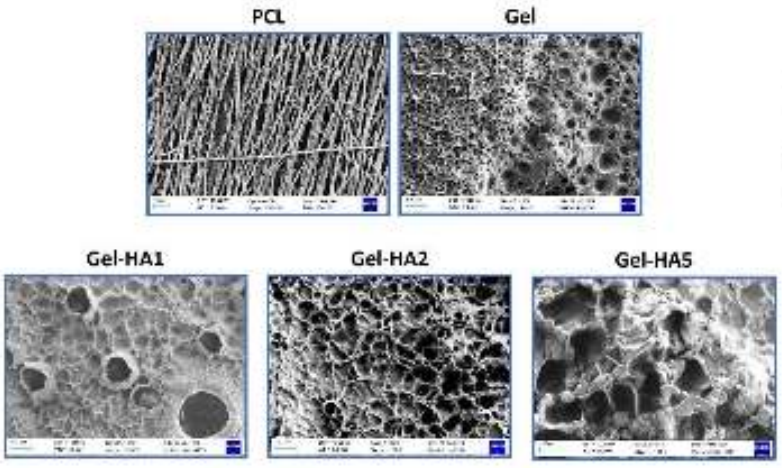
Introduction:

Around 19% of the global population suffers from severe periodontal disease, which poses a significant burden on healthcare systems like the NHS, costing approximately £3.6 billion annually. Current treatments, such as surgical implants and allografts, have limitations like donor tissue scarcity and potential risks of disease transmission or rejection. Guided bone regeneration (GBR) has emerged as a promising alternative, utilizing barrier membranes to facilitate tissue growth. Our objective is to develop a bilayer scaffold for GBR treatment. The scaffold comprises a porous gelatin hydrogel layer infused with hydroxyapatite (GelHA) for mechanical strength and loaded with Simvastatin to enhance bone formation. A polycaprolactone (PCL) membrane will be electrospun onto the GelHA layer, serving as a barrier to prevent tissue migration. We anticipate that this Gelatin-PCL structure will effectively stimulate osteogenesis in periodontal defects, paving the way for potential clinical applications.

Materials and Methods:

Initially, the hydrogel is fabricated using the freeze-drying technique. Gelatin solution is thoroughly mixed with hydroxyapatite until homogenized, forming a gel upon resting. This gel is then frozen and subjected to a freeze-drying process, resulting in a porous scaffold with a pore size ranging from 100 to 300 µm. Subsequently, the scaffold is loaded with a solvent containing dissolved simvastatin. Following the hydrogel preparation, the PCL membrane is electrospun onto the GelHA hydrogel. PCL is loaded into a syringe and applied to the scaffold positioned on the collector. The application of high voltage induces electrostatic forces, stretching the PCL into fibers along the scaffold. The scaffold's physicochemical properties are assessed using SEM, FTIR, DSC, and DMA. Additionally, the release of simvastatin from the GelHA is investigated. Finally, the pre-osteoblast mouse cell line MC3T3-E1 is cultured, seeded onto the scaffold, and subjected to Live-Dead and ELISA assays to evaluate cell viability and proliferation.

Results and Discussion:



The results demonstrated that the nanofiber structure of PCL, along with the porous architecture of Gel and HA-loaded Gel at concentrations of 1, 2, and 5 w/v%, exhibited interconnected pores.

Figure 1. Morphology of PCL fibers, Gelatin and HA loaded Gelatin with various concentrations (1, 2 and 5 w/v%), observed by SEM.

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MANUFACTURING ELECTROSPUN POLYCAPROLACTONE FIBRE SCAFFOLDS FOR LIVER TISSUE ENGINEERING

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Introduction

Electrospinning allows for the fabrication of 3D scaffolds with a tailorable nano-fibrous structure that can be designed to mimic the microenvironment of the extracellular matrix for use in tissue engineering [1]. Cell spheroids are proven to accurately mimic *in-vivo* microenvironments and provide more physiological relevance than a traditionally grown 2D mono or co-culture [2,3]. Electrospun fibre architecture has shown to influence cell the behaviour and growth of hepatocytes and there is a lack of recent literature on its effect on hepatic based spheroids [1]. This study aims to compare the function and behaviour of hepatocytes cultured as 2D cultures and 3D spheroids seeded to a polycaprolactone (PCL) scaffold for use in Human liver tissue engineering.

Materials and Methods

Scaffolds were fabricated from a solution of 19 w/v% PCL in Chloroform:Methanol (5:1) that was Electrospun (IME Technologies) under various conditions to produce fibres of random and aligned orientation. Random and aligned fibres were obtained with a mandrel rotation speed of 250 RPM and 1800 RPM respectively. A preliminary assessment of scaffold characteristics was conducted with scanning electron microscopy (SEM), water contact angle (Cell Scale), tensile testing (Instron 3367) and 2D fast Fourier transform image analysis (MATLAB). We aim to seed scaffolds with hepatocytes (HepG2 and Human umbilical vein endothelial cells) that have been mono and co-cultured in systems to form 2D cultures and 3D spheroids. Spheroid culture methods will be assessed in systems such as with the hanging drop method and in microwell arrays. A complete assessment of mechanical characteristics, cell viability and function via biochemical, histological and gene expression will be conducted spanning 14 days on the seeded scaffolds.

Results and Discussion

Initial results indicate that PCL scaffold morphology can be produced in a stable manor with continuous fibre diameter size across the material surface; Mechanical characterisation and SEM investigations reveal a variance in fibre diameter, water contact angle and Young's modulus between scaffold groups (Tab 1, Fig 1).

	Random	Aligned
Scaffold thickness (µm)	587 ± 41	403 ± 25
Fibre Diameter (µm)	4.84 ± 0.27	4.64 ± 0.21
Water contact angle (°)	131.78 ± 6.60	129.97 ± 8.41

Table 1: Preliminary results of PCL scaffold thickness, fibre diameter and water contact angle for each architecture. N ≥ 3 presented as mean ± standard deviation.

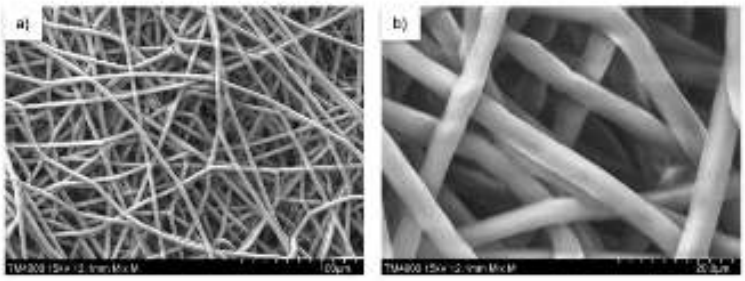

Figure 1: SEM images from preliminary morphology assessments of a PCL scaffold with randomly orientated fibres.

Conclusions

This initial development in material fabrication will pave the way for further investigations into spheroid culture on electrospun fibres and how they could be optimised to provide a novel environment for hepatocyte culture. This study's outputs could identify new methods in the development of tissue engineered Human liver models.

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Acknowledgements: EPSRC studentship EP/W524384/1 and MRC grant MR/L012766/1

DEVELOPMENT OF A NOVEL PLANT-DERIVED POLYSACCHARIDE-BASED HYDROGEL FOR BONE TISSUE ENGINEERING.

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Introduction:

The healing of critical-size bone defects is a major challenge in bone tissue engineering. The controlled delivery of growth factors (GFs) has shown the potential to induce tissue regeneration. However, high doses of GFs are required to obtain significant improvement in clinical settings, leading to severe side effects. Herein, a plant-derived polysaccharide (acemannan, ACE), that possesses immunomodulatory properties and promotes proliferation, has been incorporated in PEG-based hydrogels to develop novel 3D carriers with tuneable physicochemical properties. Such hydrogels enabled the controlled delivery of GFs, promoting the osteogenic differentiation of mesenchymal stem cells.

Methodology:

Biological activity of acemannan was tested in 2D by viability, adhesion, and osteogenic differentiation of human mesenchymal stem cells (hMSCs). Afterwards, PEG hydrogels incorporating acemannan (0.1% or 0.2%) were developed. PEG-MAL polymer was crosslinked with PEG-diSH and a protease-degradable peptide (VPM), physically entrapping the ACE into the PEG network. Fibronectin fragment (FNIII_12-14) produced by competent bacteria was used to functionalise the PEG network with cell adhesive and GF-affinity motifs, also allowing an efficient incorporation of BMP-2 within the network. The mechanical properties of the hydrogels were measured by rheology; and swelling, degradation and ACE retention were also tested. Then, the release of BMP-2 encapsulated in the hydrogel was evaluated. The biological response of these hydrogels was assessed through viability, proliferation, and differentiation studies in vitro.

Results and Discussion:

The biological characterization in 2D showed the positive effect of ACE on both viability and adhesion of hMSC. Moreover, Alizarin red staining assay and OCN staining by In-Cell Western demonstrated the capacity of ACE to promote hMSCs osteogenic differentiation.

Then, protease-degradable PEG/ACE-based hydrogels were engineered. Such hydrogels presented tuneable physicochemical properties, as shown by swelling, degradation, and rheology measurements, while retaining the ACE in the hydrogel network. Fibronectin fragments with affinity for GFs were successfully incorporated to increase the sustained release of the GF. The biological characterisation of such hydrogels showed the capacity of ACE to promote hMSCs osteogenic differentiation, as demonstrated by the ALP activity and qPCR results.

Based on the observed results, it is suggested that acemannan can function as a bioactive compound. Furthermore, when combined with tuneable PEG-hydrogel, the results suggest that this system has the potential to be a novel candidate for bone application.

Conclusion:

Biological characterisation in 2D demonstrated the potential of ACE to promote hMSCs osteogenic differentiation. Such results were then translated to 3D, where degradable PEG hydrogels loaded with ACE and BMP-2 also triggered hMSCs osteogenic differentiation, suggesting the potential of this system as a promising approach to promote bone tissue regeneration.

3D BIOPRINTED VASCULAR NETWORK WITH ALGINATE-COLLAGEN BASED BIOINK TO MONITOR ANGIOGENESIS MEDIATED EXTRACELLULAR REMODELLING

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Introduction: Angiogenesis plays a pivotal role in development and tissue growth, as well as in pathological conditions such as cancer. Being able to understand the basic mechanisms involved in the vascularization of tissues and angiogenic network formation provides a window to advance the development of in vitro tissue models and enhance tissue engineering applications. We aim to leverage novel microfluidic-based three dimensional (3D) bioprinting technology and alginate-collagen type I (AGC) bioink, to develop a 3D bioprinting strategy to enable the biofabrication of complex angiogenic networks.

Materials and Methods: Bioactive components (laminin, gelatin, fibronectin and combined) were added to alginate-collagen based bioink (AGC) matrix to form blended bioinks. simian vacuolating virus 40 (SV40) transformed adult rat brain endothelial cell (SV-ARBEC) cells were trypsinized, centrifuged and re-suspended in one of the five AGC bioinks to reach a final concentration of 15 million per mL. Structures were printed with Aspect Biosystems RX1 bioprinter with microfluidic based DUO printhead (Figure 1a). The printed constructs were immersed in feeding media and incubated at 37°C with 5% CO_2 for up to 30 days. Cell viability assay were employed at different time points. Actin filament and cytoskeleton organization within the 3D structure were visualized with phalloidin staining using Zeiss LSM-410 (Thornwood, NY, USA) confocal microscope. The unstained collagen fibrillary structure was imaged by SHG microscopy using multiphoton microscopy platform utilizing a femtosecond laser (Insight DS+, Spectra Physics, USA).

Results and Discussion: Over the 14-day culture period, all bioinks retained high cell viability (~70%) and facilitated SV-ARBEC proliferation while maintaining hydrogel fiber diameter integrity (Figure 1b). Cytoskeletal orientation mapping analysis demonstrates high degree cytoskeleton alignment along the hydrogel fiber deposition direction with extensive sprouting and stable interconnected endothelial network formation within 30 days (Figure 2). The collagen type-I in AGC bioink underwent remodeling with decreasing in collagen bundle length and 4 folds increase in average thickness from first day (Figure 1c).

Conclusion: We found that all AGC-enriched bioink preparations (AGC, AGC-L, AGC-G, AGC-F and AGC-LGF) supported SV-ARBEC cell adhesion, survival and proliferation. All formulations also facilitated induction and progression of angiogenesis and vasculogenesis events leading to robust vascular network formation over long term cultures. The intrinsic collagen type I fibers in the AGC bioink, acts as a structural reinforcement to enhance the physical and mechanical properties of the bioprinted construct. These intrinsic collagen type I fibers facilitated subsequent SV-ARBEC mediated collagen fibrillogenesis and stimulating cell remodelling and collagen deposition. In conclusion, these results demonstrate the great potential of 3D bioprinting as superior alternative in generating functional tissues with robust vascularized network formation.

Abstract theme: Biomaterials

FROM NATURE TO TREATMENT: A BIO-HYBRID HEMODIALYSIS MEMBRANE

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Introduction

Chronic kidney disease is identified by a gradual loss of kidney capabilities, which can extend over several months to years. These diseases are an escalating concern for public health and are projected to rank as the fifth leading death cause by the year 2040 (1). For individuals at the terminal stage of kidney disease renal replacement therapies such as hemodialysis (HD) become essential.

The composition of dialyzer membranes for HD is based on synthetic polymers with improved biocompatibility and control over pore size. The exploration of natural materials like chitosan highlights the continuous efforts to enhance the sustainability of dialyzer membranes and maybe their reutilisation (2).
This research introduces a novel investigation into the extraction and use of chitosan from insects (*Tenebrio molitor*) for HD membrane design.

Materials and Methods

Methods for efficient extraction were developed, taking between 6 to 12 hours, yielding chitosan with physicochemical and bioactive properties regarding Fourier transform infrared spectroscopy (FTIR) with deacetylation degrees and antimicrobial properties. Furthermore, this research developed a chitosan-based membrane (CH-M) specifically designed for HD, moving into an HD simulation system focusing on diffusion and retention permeability assessments of biochemical blood indicators.

Results and Discussion

Chitin was first effectively separated from *T. molitor* through deproteinization and demineralization processes, achieving about 5% yields. Chitosan was then produced from this chitin through a sped-up process, achieving yields ranging from 65.0 ± 0.8% to 79.3 ± 0.8%. Structural similarities to commercial chitosan from crustaceans were verified through FTIR with deacetylation degrees found to be between 73 - 75%. The antimicrobial and antioxidant properties of the *T. molitor*-derived chitosan were tested, showing inhibition of clinical pathogenic microorganisms with minimum lethal concentrations ranging from 2 to 8 mg/mL.

The *in vitro* test of CH-M's permeation of urea and albumin demonstrated that urea could permeate more than 70% while albumin was completely retained.

Conclusions

This detailed exploration and creation of a bio-inspired alternative for HD emphasize sustainability and innovation. However, it also notes the necessity for further investigations into biocompatibility, treatment duration simulations, porosity, permeability, and hemocompatibility testing.

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OPTIMISING EXTRACELLULAR MATRIX ANALOGUES FOR 3D MODELLING OF OSTEOSARCOMA

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Abstract theme: Bioengineered Models, Biomaterials

Introduction: Osteosarcoma (OS) is an aggressive primary malignant tumour mainly arising in the metaphysis of the long bones of extremities. Despite the advances in oncologic drug discovery, patient outcomes have not improved significantly for the past 30 years[1]. To discover new OS therapies and study cancer progression, more physiologically representative models need to be developed. Three-dimensional (3D) *in vitro* models better recapitulate the complexity of tumour microenvironment compared to 2D models by incorporating critical physical matrix cues. The development of more physiologically relevant OS models requires substrates that can recapitulate the mechanical properties of the physical microenvironment. In this study, we used cross-linked alginate hydrogels (AHs) and poly(lactic acid)-based microparticles to act as substrates for 3D OS cell culture to mimic the various aspects of the extracellular matrix of this bone cancer. We investigated the effects of 3D topographical features and substrate stiffness on OS cell viability and 3D cell-substrate morphology.

Materials and Methods: Alginate hydrogels of varying stiffnesses were fabricated by crosslinking 1% (w/v) alginic acid with 1% (w/v) calcium chloride. Stiffness was varied by changing crosslinking period: soft (5 minutes) versus stiff (15 minutes) before removing the cross-linking agent. In addition, microparticles of varying surface topographies (dimpled and smooth microparticles) were fabricated as described before[2]. To assess OS cell-material interactions, two established osteosarcoma cell lines, MG-63 and U2OS (representing fibroblastic and epithelial phenotypes, respectively) were cultured on microparticles for 24 hours, and in AHs for 72 hours. Their viability was measured using the ReadyProbes™ Cell Viability Imaging Kit ThermoFisher Scientific). Automated image analysis was carried out using CellProfiler.

Results and Discussion: Varying the 3D topographies showed a significant impact on OS cells, with low U2OS cell viability on dimpled microparticles and in 3D non-adherent controls, unlike those cultured on smooth microparticles (Fig. 1A, B). In addition, notable differences in cell-aggregate morphology were observed between the different samples, where U2OS cell-microparticle aggregates were significantly larger in size than MG63 ones (Fig. 1B). High viability was observed on both soft and stiff AHs, with viable MG-63 cells cultured in soft AHs representing 88.6% of total cells relative to 91.8% viability within stiff AHs (Fig. 1C).

Conclusions:
We demonstrate that cross-linked alginate hydrogels were biocompatible with OS cell lines under different conditions of stiffness. Responses of the two cell lines to topographically textured microparticles showed clear differences in OS cell-microparticles viability and aggregate morphology, suggesting that the phenotypic differences play an important role in determining OS cell response to different topographical features.

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Acknowledgements
This work is supported by a Springboard Award [SBF008\1057] from the Academy of Medical Sciences.

Figures

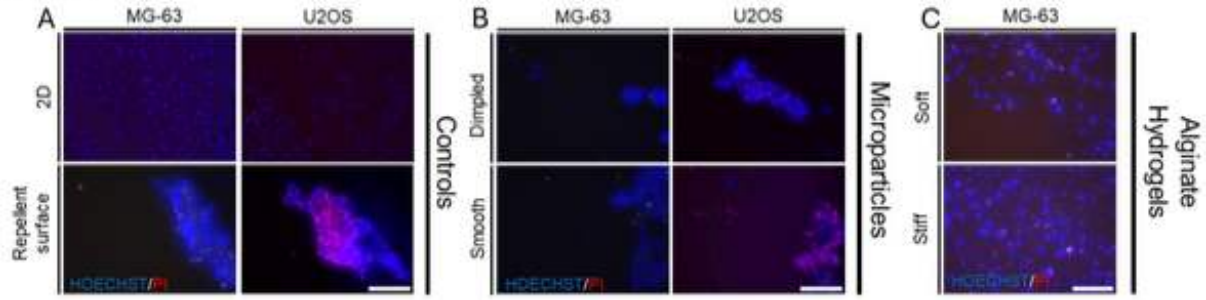

Fig 1. Representative fluorescence microscopy images of MG-63 and U2OS cells cultured as (A) 2D and 3D non-adherent cultures, and (B) 3D cultures on dimpled and smooth microparticles. (C) MG-63 cells showed high viability when cultured within soft/stiff AHs. All nuclei are stained in blue (Hoechst 33342), with nuclei of cells undergoing necrosis being stained in red (Propidium Iodide). Scale bar: 250 μm.

PREPARATION AND CHARACTERISATION OF SYNTHETIC HYDROXYAPATITE USING A MICROWAVE-ASSISTED METHOD FOR BIOMEDICAL APPLICATION

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Hydroxyapatite (HA or HAp) is a well-known biomaterial. Calcium and phosphate are the main building blocks of natural bone's hydroxyapatite, which has similar properties to synthetic hydroxyapatite (HA), especially when it comes to chemical composition. Therefore, it possesses bioactivity, excellent biocompatibility, commendable osteoconductivity, the absence of toxicity, and inflammatory reactions, making it a suitable synthetic alternative material for bone regeneration. Various techniques can be employed to synthesise HA, including solid-state reaction, hydro- and solvothermal methods, precipitation, combustion, the use of micro-emulsion, ultrasonic synthesis, and bionic approaches. The diverse techniques employed for synthesising have an influence on the various shapes and sizes of HA, which can have repercussions on its chemical, physical, and mechanical characteristics.

Alternatively, microwave synthesis is another intriguing approach for producing HA. The microwave approach differs from other standard ways for HA preparation by aqueous synthesis with a highly uniform spatial temperature distribution under high pressure. Hence, this research focuses on employing the microwave technique to synthesise HA, with the primary aim of characterising the chemical and physical attributes of this substance.

In this study, we will prepare and conduct experiments on controlling microwave conditions using two primary variables: temperature and ramping time for synthesis. The sample will then undergo a calcination process at 550 degrees Celsius. The physical properties of the sample will be characterised using Scanning Electron Microscopy (SEM) or Transmission Electron Microscopy (TEM) to analyse its structure, surface, and size. The chemical properties were analysed by X-Ray diffraction (XRD) and X-ray fluorescence (XRF), to determine the crystallinity and elemental composition. Cell proliferation and cytotoxicity procedures are used to assess a significant factor in the self-supporting biocompatibility of biocompatible medical devices.

In the future, a composite material will be developed by combining hydroxyapatite (HA) and biopolymer for the purpose of creating bone substitute scaffolds. These materials will then undergo testing to evaluate their impact on bone regeneration by assessing osteoblast proliferation.

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JELLYFISH COLLAGEN FILLED TITANIUM MATRIX FOR LARGE BONE DEFECT REPAIR

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Abstract theme: Biomaterials

Introduction

Large bone defects can be caused by trauma, disease, surgery, or tumour resection where the bone is unable to heal as normal due to the size of defect or fracture. Autografts and allografts (with/ without non-biological materials, such as metallic fixtures) are the current gold standard but these treatment methods come with various problems from limited supply to donor site morbidity and immune rejection [1-2].
To understand and resolve this clinical problem, regenerative medicine approaches such as tissue engineering and biomaterial use, have been widely studied and practised. A more recent type of collagen that has emerged is jellyfish collagen (Jellagen), which is collagen type 0, non-cytotoxic, and biocompatible and more readily available than other types of collagen. Titanium (Ti) and its alloys are clinically established in bone implants. In this project, we are investigating how the combination of these two materials to form a hybrid jellyfish collagen – Ti scaffold can provide a natural 3D microenvironment for bone formation.

Materials and Methods

Ti (Ti-6Al-4V) matrices were infilled with jellyfish collagen and freeze-dried. Either SAOS-2 cells or human adult osteoblast cells were seeded per scaffold for the following time-points: Day 1, 3, 7, 14, 21.
Established quantitative and qualitative methods such as Live/Dead® Viability/Cytotoxicity Kit, PrestoBlue Viability Assay, scanning electron microscope (SEM), immunostaining (IF) (DAPI, Phalloidin and Osteopontin) and Alkaline Phosphatase (ALP) assay have been used to determine whether the proposed hybrid scaffold supports osteoblast viability, proliferation and differentiation.

Results and Discussion

The viability assay showed that the hybrid scaffolds maintain cell growth throughout the time-points. Cell proliferation was seen through the Live/Dead images, as seen below in Figure 1. The different cell morphologies were also seen based on the location of the cell-to-material interactions. This was further seen in the IF images, where bone cell attachment to the materials and interface was visible.

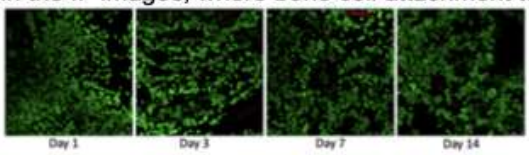

Figure 1. Confocal laser scanning microscopy visualization of Live/Dead staining of SAOS cells on hybrid scaffolds at Day 1, 3, 7, 14. Viable cells in green / dead cells in red. (Magnification of 10x)

The SEM images showed the porous structure of the samples and the different surface morphologies. They also showed the many bone cells present within the hybrid scaffold, with adherence to the Ti and collagen across 14 days.

Conclusions

The viability results showed that the hybrid scaffold supports proliferating bone cells long term, further confirmed by Live/Dead images showing viable cells throughout the scaffolds. The SEM images showed the cell morphology and strong attachment to the different materials. This was also seen in the IF images where cells are present on and around the Ti matrix, around the freeze-dried collagen pores and in the collagen-Ti interface, which is of great interest. With these promising results, the next step would be to assess the drug-delivery capability of the scaffold while promoting bone formation.

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Acknowledgements This work was supported by Jellagen Ltd and the Engineering and Physical Science Research Council (EPSRC) via DTP case programme (Grant No. EP/T517793/1).

LIGHT-RESPONSIVE ENGINEERED LIVING MATERIAL FOR LYCOPENE SYNTHESIS

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Introduction: Retinoids and their precursors form a diverse group of molecules, encompassing lycopene, β-carotene, retinal, retinol (commonly known as vitamin A), and retinoic acid. These compounds find extensive applications in biomedicine, as well as the food and cosmetic sectors, owing to their antioxidant, anti-inflammatory, and antiaging properties. Traditional extraction methods from fruits and vegetables are often laborious and expensive, resulting in degradation or oxidation of the compounds. In this scenario, *Lactococcus lactis* emerges as a promising platform to produce bioactive compounds in situ using inducible systems, such as the NICE system, which offers precise control over genetic expression to modulate the synthesis in a predictable, linear manner. Inducible systems sense different inputs, such as small- molecules, temperature, pH, or light. Our group has recently developed a blue-light inducible system for *L. lactis* [1], which paves the way to novel applications, such as the development of new smart Engineered Living Materials (ELM) able to produce lycopene to protect against the oxidative stress derived from light exposure.

Materials and Methods: To successfully produce lycopene, *L. lactis* NZ9000 was engineered with two exogenous enzymes: phytoene synthase (*crtB*) and phytoene desaturase (*crtI*), to transform geranyl-geranyl pyrophosphate (GGPP) into phytoene and further lycopene. Although this enzyme is naturally present in the probiotic, GGPP synthase (*crtE*) is also incorporated to increase the GGPP pool, thus improving the yield. In this regard, a codon optimization of *Pantoea ananas* DNA sequences, retrieved from UniProt Database, was performed. The DNA sequences were obtained from Twist Biosciences and sequentially incorporated using restriction enzymes into the pTRKH2 shuttle vector under the NICE system control, obtaining the pTRKH2-PnisA-crtEBI plasmid. On the other hand, we used the pSEUDO vector [2] to integrate the blue-light inducible T7RNAP into the bacterial genome. We replaced the PnisA with the corresponding T7 promoter, rendering pTRKH2-T7p-crtEBI, and we electroporate *L. lactis*.
We developed an ELM by combining *L. lactis* with a skin-suitable matrix (e.g., Pluronic F-127) to improve lycopene delivery and bacterial stability. The synthetic profile of *L. lactis* was assessed by culturing in GM17 medium for 3 days at 30ºC and 200 rpm, followed by EtOH:acetone:hexan extraction. The absorbance was measured in the UV-Vis spectrophotometer: i) *L. lactis* pTRKH-PnisA-crtEBI was induced with 25 ng/mL nisin, and ii) *L. lactis* pTRKH2-T7p-crtEBI was induced with blue-light. Viability and flow cytometry assays were also performed to assess the effect that the induction has on bacterial integrity both alone and when integrated in the ELM.

Results and Discussion: *L. lactis* was successfully engineered to i) produce lycopene under nisin induction using the pTRKH2-PnisA- crtEBI plasmid, and ii) the light-inducible system can be incorporated into *L. lactis*.
The organic extraction from pellets can inform about the amount of lycopene synthesized by the probiotic. The UV-Vis spectra allow the estimation of lycopene concentrations and reveals that the main molecule obtained is all-*trans*-lycopene. The probiotic can be integrated into the hydrogel to obtain an ELM, although it affects the yield of lycopene synthesis and the cell viability.

Conclusions: *L. lactis* is a promising food-grade host, Generally Recognized as Safe (GRAS) by the FDA, that can be genetically engineered to produce lycopene under nisin control and can be adapted to sense blue-light. Our work is focused on the implementation of the blue-light inducible system to obtain a probiotic able to produce lycopene, and its combination with a skin suitable hydrogel, resulting in an antioxidant smart ELM with the potential to protect against ROS produced by light exposure.

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Acknowledgements: MSCA Fellowship. UKRI Horizon Europe Guarantee Funding. EPSRC, EP/Y03029X/1

DEVELOPMENT OF DECELLULARIZED EXTRACELLULAR MATRIX SCAFFOLDS FOR THEIR POTENTIAL USE AS VALVE LEAFLETS FOR THE TREATMENT OF CONGENITAL HEART DISEASE

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Introduction

Children born with congenital heart disease (CHD) usually have a poor quality of life due to the exposure to multiple high-risk surgeries during their lifetime. Standard surgical procedures require the use of materials that are not able to grow or remodel as the child grows. The aim of this work is to develop a decellularization approach that preserves the ECM structure and composition in addition to achieving good biomechanical characteristics of decellularized extracellular matrix (deECM) biomaterials to be used as pulmonary valve leaflets for RVOT reconstruction in children suffering from Tetralogy of Fallot/pulmonary atresia.

Materials and Methods

The human amniotic membrane (AM) and porcine pericardium (PE) biomaterials were decellularized using enzymatic approach and assessed using histological (H&E, EVG, DAPI, Picrosirius Red and Alcian Blue), biochemical (DNA, GAG's, soluble collagen type I and total protein content), biomechanical (ultimate tensile strength and elastic modulus) and morphological (SEM) techniques to ensure removal of nuclear content and maintenance of the structure and composition of their ECM.

Results and Discussion

Our results showed a significant decrease in nuclear content in the deECM biomaterials compared to their native counterparts when assessed using DNA quantification. This was also qualitatively confirmed using H&E and DAPI staining. There was a good presence of soluble collagen type I in the deECM AM which was also confirmed using Picrosirius Red (using polarized light) and EVG staining as evidence for the preservation of extracellular matrix structure. Picrosirius Red staining of both deECM materials showed thick, organized collagen type I (yellowish-red colour) and thin, unorganized collagen type III (green colour). There was a significant decrease in GAG content in deECM biomaterials compared to their native counterparts however, the decellularization did not fully eliminate the GAG content in the deECM biomaterials thereby, preserving the ECM composition. This was also qualitatively confirmed using Alcian blue staining showing good presence of GAG. There was a significant decrease in ultimate tensile strength and Young's modulus in deECM biomaterials compared to their native counterparts however, the obtained results are similar to the ultimate tensile strength and Young's modulus of native pig pulmonary valve leaflets. SEM imaging of deECM biomaterials show removal of cells whilst keeping the ECM structure intact compared to their native counterparts.

Conclusions

In conclusion, the decellularization of human AM and porcine PE scaffolds was proven to be successful whilst preserving the extracellular matrix structure and composition and possession of good biomechanical properties. This approach holds promise to produce good quality of in-house scaffolds that could, potentially, be used as valve leaflets for the treatment of congenital heart disease.

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MICROSPHERES WITH PODS® ENABLING SUSTAINED SUPPLY OF BIOFUNCTIONAL PROTEIN

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Abstract theme: Biomaterials

Introduction

PODS® are polyhedrin protein based robust nano-crystals that deliver growth factors in a sustained manner, thereby obviating the need for frequent replenishment of growth factors crucial for cellular maintenance and functionality. Microspheres, traditionally employed for achieving controlled release of pharmaceuticals and bioactive agents, offer a promising avenue for sustained delivery. Alginate, recognized for its eco-friendly, biocompatible, and xeno-free properties, has been extensively studied in biomedical research, showcasing its versatility in various applications. Here, we present alginate-based microspheres encapsulating PODS®, serving as enduring reservoirs for bioactive entities such as growth factors, cytokines, hormones, and other proteins, thereby advancing both biotechnological and biomedical endeavours.

Materials and Methods

In this study, we have employed microfluidic systems to fabricate alginate-based microspheres. These microspheres have been loaded with PODS® with various cargos and their capability to release functional bioactive compounds over specific durations has been thoroughly assessed.

Results and Discussion

The encapsulation efficiency of total protein within each microsphere can be precisely modulated through the manipulation of PODS® concentration within the alginate solution. Currently, microspheres are engineered to encapsulate total protein ranging from 0.56 to 1.4 picograms per individual microsphere. These microspheres exhibit remarkable stability, demonstrating a shelf life of up to one year when stored at 4°C. Moreover, they exhibit resilience across various media conditions, retaining nearly 50% of functional protein even after a month under culture conditions, in contrast to the near complete depletion observed within three weeks in microspheres containing recombinant growth factors. Moreover, PODS® microspheres offer versatility in accommodating various payloads, including fluorescent markers, growth factors, cytokines, or customized cargoes, thereby expanding their potential applications. Furthermore, these microspheres are adaptable for incorporation into other biological matrices such as soft gels, thereby broadening their potential applications as composite scaffolds.

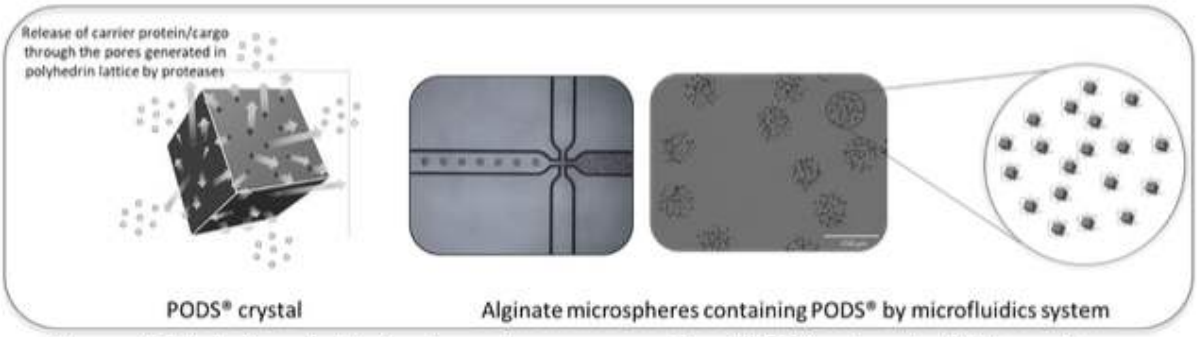

Figure 1. Fabrication of alginate microspheres encapsulating PODS® using microfluidics systems.

Conclusions

Microspheres containing PODS® represent a superior alternative to conventional microspheres containing recombinant growth factors, offering sustained protein delivery without the risk of burst release. Moreover, this material holds promise for diverse applications beyond biomedicine, including vaccine production and cultivated meat production. By adapting it as a microcarrier, it can facilitate the cultivation of large cultures of specific cells and microtissues, thereby extending its utility across various domains.

Title: Utilising the 'Design of Experiment' Statistical tool for Bioink Composition for Soft Tissue Engineering
X

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Abstract theme: Biomaterials

Introduction: The development of Bioinks for Extrusion-based 3D bioprinting presents significant challenges and opportunities in Tissue Engineering. Mechanical properties of bioinks, such as viscosity, elastic modulus, and shear-thinning behaviour, need to be suitable as they are crucial for successful 3D bioprinting. By characterising these properties through formulation and processing, bioinks can be tailored to mimic native tissues and support cell viability, proliferation, and tissue regeneration within printed constructs. The integration of Rheology and Design of Experiment statistics (DOE) offers a systematic approach for developing bioink formulations. Rheology characterises the bioink properties, while DOE provides a methodical platform to explore the bioinks' properties and identify optimal conditions. By combining these methodologies, this study investigates the development of a bioink for Soft-tissue 3D-bioprinting with tailored properties to enhance printability, structural integrity, and cellular viability.

Materials and Methods: Minitab® 21 was utilised to format Mixture Design of Experiment and a 2-level Factorial Design of Experiment using 4 animal-free components to create the bioink mixture. CELLINK SKIN bioink was used as a reference viscosity point to establish the target viscosity of the mixture. Samples were prepared in accordance with the DOE outputs. These samples were then subjected to an Isothermal Viscosity Test using the MCR-92 Rheometer at a high shear rate of 80 s-1 at 37°C. Using Minitab® 21, the Capability Analysis test was performed on the rheology data, with a upper and lower boundaries of +/-10% error either side of the desired target. The test also produced a potential capability value (Cpk), which evaluates the capability of the process. The CpK benchmark is set at 1.33.

Results:

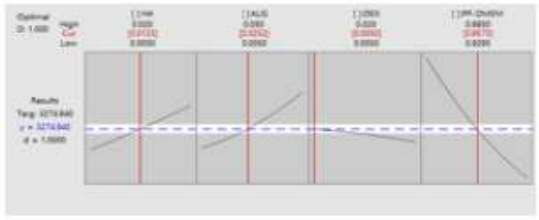

Figure 1 - Response Optimisation of Bioink Designed by the Mixture DOE

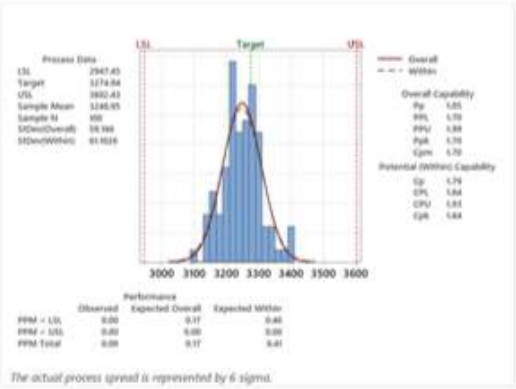

Figure 2 - Capability Analysis of DOE process for Bioink Development

Discussion & Conclusions: Utilising the DOE tool within this methodology streamlines the process by eliminating time-consuming trial and error methods and allowing for the precise design of an optimised bioink tailored to specific targets. Incorporating the use of 4 animal-free components enhances the versatility of the bioink, mimicking the natural cellular microenvironment crucial for soft tissue engineering. By utilising a commercially available bioink (CELLINK SKIN) as a baseline for viscosity, assumptions can be made to predict printing parameters, facilitating their application during the printing process. The DOE tool optimised the process for bioink formulation at a 100% viscosity match to the reference viscosity and passed the benchmark in the capability analysis, producing a Cpk of 1.64.

AcknowledgementsThe authors would like to acknowledge the generous contribution of Anton Paar. Also, to acknowledge the financial contribution from the NHS, Health Education England Southeast for funding the project, and to ARC/KSS for funding the PhD Scholarship within the Faculty of Medicine, Health, and Social Care at Canterbury Christ Church University.

REGENERATION OF THE ROTATOR CUFF ENTHESIS THROUGH BIOFABRICATION

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Background: One in five people are affected by shoulder pain at any given time. For about 40% of the cases, tears in the rotator cuff are the cause. Surprisingly, keyhole surgery remains the most effective approach to repair the degenerated tendons, even though the success rate is suboptimal, as proven by incomplete healing in 10-70% of cases.[1,2]

Objective This project aims to produce an acellular scaffold implant from bacteria-derived biomaterials to improve the healing of a torn rotator cuff tendon. Moreover, the scaffold may serve as a more suitable platform for the study of fundamental mechanisms of tissue regeneration or disease progression in the human shoulder. This main objective is broken down into several goals:

1. Investigating natural materials with suitable physical, chemical, and biological properties for native tissue mimicry
2. Exploring biofabrication strategies for processing these materials into 3D scaffolds
3. Designing, manufacturing, and *in vitro* testing of hybrid scaffolds
4. Assessing effectiveness and improving scaffold performance in *in vivo* models

Methodology: A library of natural materials will be established since they can meet the key desirable properties including biocompatibility, biodegradability as well as antimicrobial, anti-inflammatory and tuneable mechanical properties. Polyhydroxyalkanoates (PHAs) will be the initial focus of the material search due to their property variability, facilitated by their biosynthesis in a fermentation process. Next, the selected materials will be processed into bi-layered/multi-material scaffolds with multi-scale imprinted functional gradients. Melt electrospinning direct writing (MEDW) is the preferred method, but others may be explored depending on the initial outcomes. To find the ideal scaffold nanostructure, *in vitro* cellular assessments will be carried out first with differentiated human cell lines, i.e., of osteoblasts, chondrocytes, and tenocytes. Later, stem cell differentiation should be triggered by structural cues in the optimised scaffold. Once achieved, *in vivo* tests in animal models such as mice or rabbits will clarify important aspects like patient stem cell infiltration and functional tissue formation. If promising, the clinical translation of our findings will be promoted through *in vivo* testing with human patients. In collaboration with our industrial partner, strategies to accelerate the commercialization of our products will be explored to achieve a positive socio-economic impact.

Acknowledgements:

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COMPARISON OF PRIMARY SUBMANDIBULAR GLAND EPITHELIAL CELLS AND HSG CELL LINE COMPATIBILITY WITH ANTIOXIDANT SCAFFOLDS

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Introduction

Radiation treatment for head and neck cancer not only targets the cancer cells, but also influences the surrounding tumor microenvironment (TME), including healthy cells. Such changes include increases in inflammatory responses, fibrosis, and oxidative stress, indicated through excess levels of reactive oxygen species (ROS). Antioxidants have been used to mediate these excess levels of ROS. Retinyl acetate, a derivative of vitamin A, is one example of antioxidant that can be used in such a manner (1).

Previous work has shown that retinyl acetate scaffolds are sufficient in supporting the growth and survival of human submandibular gland (HSG) (HeLa derivative) cells, and produced an antioxidant effect (2). Herein, we investigate the compatibility of the antioxidant scaffolds with primary mouse submandibular gland epithelial cells (SGECs) and compare their responses to those of the HSG cell line.

Materials and Methods

Retinyl acetate (RA) containing scaffolds were fabricated via electrospinning 0% or 0.5% (w/v) RA with 10% (w/v) polycaprolactone (PCL) dissolved in hexafluoroisopropanol (HFIP). Characterisation of the fibres included scanning electron microscopy (SEM) analysis for fibre diameter and morphology, and tensile testing. Scaffolds were seeded with SGECs, isolated from enzymatically-digested C57BL/6 mouse tissue and grown to confluency, and cultured for a period of seven days. During this period, cell viability and DNA quantitation were measured. The same protocol was followed using HSG cells and the results assessed to provide a comparison between primary SGECs and HSG cell line responses to the scaffold.

Results and Discussion

SEM analysis confirmed that both scaffold groups consisted of smooth and randomly aligned fibres (Figure 1), with fibre diameters of 1.29 ± 0.08 μM and 1.25 ± 0.08 μM for 0% and 0.5% RA scaffolds, respectively. Mechanical testing showed that the scaffolds produced were consistent with previously published work (2). Cell viability and DNA quantitation assays showed that scaffolds were capable of supporting the survival and proliferation of both primary and HSG cells.

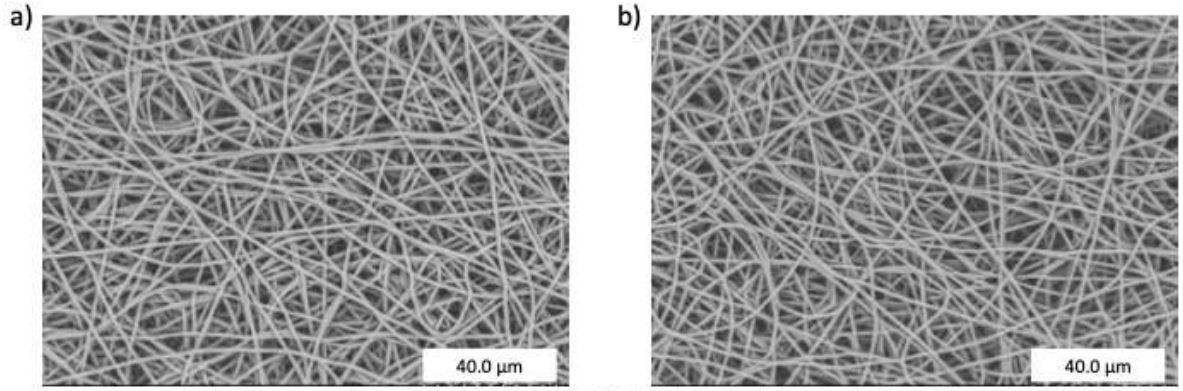

Figure 1 SEM images (x2000 magnification) of (a) PCL only fibres and (b) 0.5% RA fibres

Conclusions

Overall, it was shown that both primary mouse SGECs as well as the HSG cell line were capable of survival and proliferation on the electrospun scaffolds, with comparable results. These initial results, particularly the compatibility between the scaffolds and the primary cells, provides evidence to encourage further investigation into the use of such antioxidant biomaterials as viable therapeutic approaches.

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Acknowledgements

This work is funded by EPRSC studentship EP/T517884/1 and MRC grant MR/L012766/1.

INFLUENCE OF N-ACETYLCYSTEINE-LOADED PCL FIBRES ON OXIDATIVE STRESS FOR CARTILAGE TISSUE ENGINEERING

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Introduction

More than 250 million of the world's population is affected by osteoarthritis (OA) which causes disruption in cartilage integrity.[1,2] Oxidative stress resulting from reactive oxygen species (ROS) is one of the main factors that could cause cartilage degeneration. Antioxidants are known to reduce or prevent cartilage damage by fixing redox imbalance caused by ROS.[3] N-acetylcysteine (NAC) is an antioxidant that can participate in the regulation of oxidative stress through the thiol or indirectly cysteine group.[4] Recently, studies have been conducted showing that the inclusion of antioxidants in electrospun scaffolds supports cartilage regeneration.[5] In this study, NAC was included with the polycaprolactone (PCL) electrospun fibers. The mechanical properties, antioxidant activity, and effect of these scaffolds on chondrocytes were investigated.

Materials and Methods

PCL was dissolved in hexafluoroisopropanol (HFIP) to get a concentration of 16% (w/v). This solution was used as the control group. NAC at 0.5% and 1% concentration was prepared with 16% PCL solution. Each solution was transferred to a syringe and electrospun at 1ml/h flow rate in the total volume of 4 ml to get randomly aligned fibers. Scaffold characterization was performed using scanning electron microscopy (SEM) imaging, tensile testing, and compression testing (Instron 3367). 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydrogen peroxide assays were used to determine the antioxidant properties of the scaffolds. Bovine chondrocytes were cultured on the scaffolds to evaluate the viability of the cells.

Results

PCL fibers containing 0.5% and 1% NAC were successfully fabricated using electrospinning techniques (Figure 1). While the control group achieved the highest fiber diameter of 1.94 µm, the values obtained by the NAC-containing scaffolds were lower with 1.61 µm and 1.60 µm (Table 1). NAC-loaded scaffolds supported chondrocyte survival and proliferation.

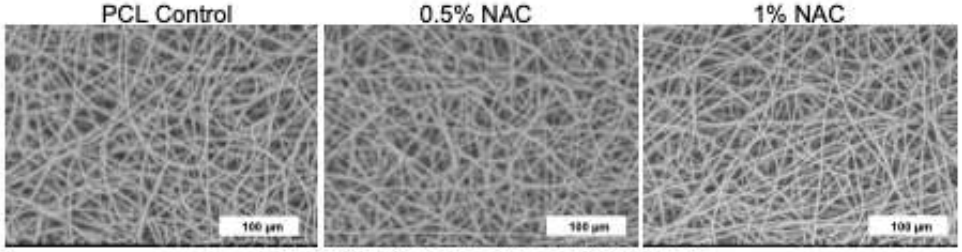

Fig. 1: SEM images of the scaffold groups.

	Control	0.5% NAC	1% NAC
Fiber Diameter (µm)	1.94 ± 0.19	1.60 ± 0.18	1.61 ± 0.20
Tensile Modulus (MPa) (0-5% Strain)	34.16± 6.28	35.68 ± 10.79	16.63 ± 2.49
Compressive Modulus (MPa) (0-5% Strain)	0.66 ± 0.73	0.52 ± 0.55	0.31 ± 0.23

Table 1: Fiber diameters and mechanical testing data of scaffold groups.

Discussion & Conclusions

The results show that ROS scavenged was achieved with the NAC scaffolds compared to the control. In addition, NAC containing scaffolds did not have a negative influence on the survival and proliferation of chondrocyte cells. This study highlights the potential use of NAC in cartilage tissue-engineered scaffolds.

Acknowledgements

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NOVEL BIOMIMETIC CELL-AIDED SCAFFOLD FOR SKIN TISSUE ENGINEERING

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Introduction: The skin, being the largest organ in the human body, plays a vital role in acting as a protective barrier. Therefore, it is imperative that wounds heal promptly to safeguard the underlying tissues. However, severe burns and medical conditions such as diabetes can disrupt the wound-healing process (Ahmad et al., 2021). Skin grafts are frequently employed for the purpose of replacing and regenerating skin (Negut, Dorcioman and Grumezescu, 2020). However, certain types of grafts, such as xenografts, can potentially give rise to clinical complications, due to the occurrence of immunological rejections (Nikolova and Chavali, 2019). To address this issue we have fabricated scaffold dermal scaffolds from cells-derived ECM that accurately emulate the physiological and structural properties of native human skin.

Material and Methods: To achieve this, we cultivated layers of human dermal fibroblasts (HDF) that were superimposed with human epidermal keratinocytes (HaCaT), with the option of incorporating collagen between the layers. Furthermore, the bilayer underwent decellularisation using an enhanced protocol, followed by an evaluation of its physical and biological characteristics through analysis of degradation, swelling ratio, and porosity. The decellularised scaffolds were reseeded with cells to assess their compatibility and implanted in the chorioallantoic membrane to examine angiogenesis.

Results and Discussion: The study involved the development of functional and durable cell-aided scaffolds through the utilisation of the cells and collagen. Based on the results of the physical tests, it was observed that the swelling ratio of the scaffolds reached 81.96%± 2.39% when the control was 44.89%± 1.92%. The degradation ratio of the scaffolds were significantly higher ($p<0.05$) in comparison to the native skin. The porosity percentage exhibited a similar trend between the two. The findings of this study also demonstrated that the scaffolds facilitated cellular adhesion and integration while also stimulating angiogenesis.

Conclusion: In summary, the study showed promising results for bilayered scaffolds as viable substitutes for human skin regeneration scaffolds. Despite some differences in physical characteristics compared to native skin, the scaffolds demonstrated compatibility with cell reseeding and promoted angiogenesis, indicating their potential for clinical applications in wound healing and tissue regeneration.

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IRON OXIDE NANOPARTICLES FOR BIMODAL HYPERTHERMIA COUPLED WITH BIOPHYSICAL AND *IN SILICO* EVALUATION WITH HUMAN HEMOGLOBIN

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In recent years, magnetic nanoparticles (MNPs) have captivated the biomedical community due to their diverse physiochemical properties and diagnostic applications. They can be employed as therapeutic agents in magnetic hyperthermia, photothermal therapy, photodynamic therapy, and magnetically targeted drug delivery. In this work, iron oxide nanoparticles were synthesised through the thermal decomposition method and then coated with PAA for organic to aqueous phase transformation. The synthesised nanoparticles were then characterised by TEM, FESEM, DLS, XRD, and VSM. To determine the potential applications for these nanoparticles, we explored two avenues. Firstly, we investigated the laser (PTT) and magnetic field-induced hyperthermia (MHT) properties of PAA-functionalized iron oxide nanoparticles (PAA-IONP). Our *in vitro* studies revealed that PAA-IONP can serve as a potential photothermal agent as it shows low dark and significant laser induced toxicity. Secondly, we examined the mechanistic interactions of PAA-IONP with human haemoglobin via spectroscopic techniques such as UV-Visible spectroscopy, fluorescence spectroscopy, circular dichroism and probed into the interactions via molecular docking. Blood plasma is essential for the delivery and targeting of medication molecules, and biological interactions between the potent drug/nanoparticle and blood proteins are critical for effective therapeutic use. From these studies, we demonstrated successful binding between nanoparticles and haemoglobin.

DOES GDF11 AFFECT THE IMMUNOMODULATORY PROPERTIES OF OLDER AND YOUNGER DONOR HUMAN BONE MARROW MESENCHYMAL STROMAL CELLS?

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Introduction

With an ageing population, cell-based therapies aimed at reducing both inflammation as well as promoting tissue repair without cytotoxic side effects is a current key goal for clinical therapy. Human mesenchymal stromal cells (hMSC) are a key progenitor for this approach; however, they are a rare population of cells in the body making ex vivo expansion essential as large numbers are required for treatment. Unfortunately, older donor MSCs (65+) typically have lower therapeutic potential, and when expanded in vitro, can become senescent, further reducing their functional properties. Therefore, new methods of expanding hMSCs whilst retaining their functional properties remains a key scientific goal. A promising candidate for improving hMSC function during laboratory expansion is growth factor differentiation 11 (GDF11). GDF11 gradually naturally declines as we age, and restoring levels of this protein has been thought to be one strategy for combating the ageing process. However, conflicting reports in the field disagree about its potential to rejuvenate hMSC from older donors.

Materials and Methods

To address the potential of GDF11 to improve the therapeutic potential of hMSC, bone marrow derived hMSC from younger (19-28) and older (65-85) donors were cultured with different doses or durations of GDF11 supplementation. hMSC immunomodulatory potential were evaluated through the ability to suppress T cell proliferation, modulate regulatory T cell maturation or the upregulation of immunomodulatory proteins (IDO1, galectin-9 and PDL-1) as well as release of soluble factors. Alterations in the actin cytoskeleton, which correlate to changes in differentiation were observed via immunofluorescence.

Results and Discussion

In this study there were no significant effects observed on improved immunomodulatory capacity of either younger or older donor hMSC suggesting that supplementation with GDF11 does not alter these important functions.

Conclusions

However, GDF11 treatment does alter other hMSC physiology processes and therefore the full effects of GDF11 remain to be elucidated for improving therapeutically functional hMSC during expansion.

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MUCUS PENTRATING NON-VIRAL GENE THERAPY FOR CYSTIC FIBROSIS VIA PULMONARY ADMINSTRATION

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Introduction: Cystic fibrosis (CF) is a genetic disease characterized by the accumulation of mucus due to CFTR gene mutations. Previous research on (GAG)-binding enhanced transduction (GET) technology has shown that these peptides improve the delivery of therapeutic cargoes into cells [1]. We have also found that coating GET/DNA nanoparticles (NPs) with polyethylene glycol (PEG) helps them move through mucus [2], which is a major barrier to inhaled therapeutics. To enhance transfection efficiency by overcoming mucus barriers and achieving improved nuclear delivery, we propose fortifying the encapsulating peptide recipe with a GET peptide that has endosomal escape capability [3]. This enhancement would further improve gene delivery efficacy to the lungs.

Materials and Methods: We prepared DNA NPs by mixing a Gaussia-luciferase (GLuc) plasmid with a combination of GET peptides (FLR, FLH, and PEG5kDaFLR). We analysed the hydrodynamic diameter, morphology, and ζ-potential of the NPs using a Zetasizer or transmission electron microscopy (TEM). To assess NP mucus diffusivities, we employed multiple particle tracking (MPT) to follow the fluorescent-tagged nanoparticles' trajectories within CF sputum. The buffering capacity and haemolytic activities of the GET peptides were evaluated to assess the peptides endosomal escape potential. *In vitro* transfection was tested on monolayers and air-liquid cultures of lung specific cells (ihbec, CuFi-5 and NuLi 1). Additionally in vivo transfection was evaluated in C57BL/6J mice after intratracheal administration of a 25-µg dose or in the sheep lung by bronchoscopic instillation of either 0.2 or 5 mg dose in 5ml volume to individual lung segments. **Results and Discussion:**

The NPs had cationic surfaces and maintained hydrodynamic diameters smaller than the average pore size of human airway mucus (< 200 nm). TEM confirmed the nanometric diameter of 45.42±7.54 nm and spherical morphology. Inclusion of PEGFLR and FLH significantly reduced ($p<0.0001$) the cationic surface to 8.17±0.35 mV compared to the parent FLR NPs (33.6±0.54 mV). Moreover PEGFLR/FLH/FLR NPs showed enhanced PBS and BALF stability in terms of no change in hydrodynamic diameter over 120 min unlike parent NPs. MPT analysis showed that the mixed peptides NPs diffused more than 10 times better in mucus compared to FLR NPs. *In vitro* and *in vivo* transfection efficiencies were significantly improved for the modified NPs compared to the original FLR NPs ($p<0.005$). In the large animal model, GLux expression in the lung segment treated with the 5 mg dose was ~4-fold and ~5-fold above the untreated level in BAL or Tissue respectively. **Conclusions:** Our results show that the inclusion of the histidine-rich GET peptide (FLH), along with the diffusive properties of PEGFLR, enhances the transfection efficiency of GET/DNA in the lung tissues following pulmonary administration. This provides a promising platform for gene therapy to address Cystic Fibrosis.

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Computational Design Of Cultured Tissue Structures With Biophysics And Machine Intelligence

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Introduction

Tools to assist mould and scaffold designs for cultured tissue growth with bespoke cell organisations are needed for applications such as regenerative medicine, drug screening and cultured meat. Computational and machine-learning models of the cell-matrix interactions vital to tissue organisation can help with this unmet need. In polarised tissues, self-organisation is driven by local interactions between cells and the extra-cellular matrix (ECM), and the onset of macroscopic forces in tethered tissues. The goal of this work is to understand and predict this self-organisation using biophysical models and machine learning. This has led to a rational design process for moulds with a high level of tissue organisation.

Materials and Methods

Several thousand moulds suitable for growing cellular hydrogels were automatically generated, including a proportion that were constrained to have high symmetries. These were used for high-throughput calculations of self-organisation in cellular hydrogels using the contractile-network dipole-orientation (CONDOR) model [1], which simulates cell-matrix interactions and tissue scale forces. We solved the biophysical model using simulated annealing. The resulting high-throughput data were used to identify promising moulds for highly aligned tissue [2] and to train an implementation of the pix2pix deep learning model [3].

Results and Discussion

We carry out validation work on CONDOR, finding close agreement with experiments on artificial neural and other tissue types. We introduce a fitness function to automatically identify moulds from the high-throughput computation with favourable properties for growing polarised tissues, and use this to find tethered mould designs, suitable for growing cultured tissue with very highly-aligned cells. We find that the machine learning technique makes excellent predictions, commensurate with the biophysical model and experiment, with a speedup of several orders of magnitude over the biophysical model. We will discuss recent approaches to using this faster approach for further improvements and new applications of mould design.

Conclusions

The CONDOR model matches well with experimental data for a wide range of polarised tissues, showing that microscopic cell-ECM dipole interactions and tissue-scale forces cooperatively drive macroscopic self-organization. We find that a CONDOR approach is suitable for high-throughput rational design of artificial tissues, predicting that two specific tethering strategies within elongated moulds lead to highly-aligned cells: (1) placing tethers within bilateral protrusions guides alignment (2) placing tethers within a single vertex reduces opportunities for misalignment [2]. Moreover, a machine-learning approach trained on this data [3] is both significantly faster than the biophysical method, opening the possibility of very high throughput rational design of moulds, scaffolds and 3D printing strategies for a range of cultured tissue applications.

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Abstract theme: Enabling Technologies
Title: Cryoprinting enables 3D printing of low viscous materials towards hydrogel-based electronic devices

Introduction

Several commonly used hydrogel bioinks, such as alginate, have a low viscosity, making it challenging to bioprint them with conventional printing techniques. Recently, cryobioprinting has been established, which enables the fabrication of cell-laden, shelf-ready tissue constructs (Ravanbakhsh et al., 2022). Additionally, this technique offers enhanced design freedom as it enables extrusion bioprinting in the Z-direction as well (Luo et al., 2022).
In general, hydrogels can be mixed with conductive polymers to stimulate cells, for example, to modulate proliferation and differentiation, thus enhancing the functionality of biomaterials (Heo et al., 2019). However, since most conductive polymeric materials have poor solubility and are mechanically brittle, it is difficult to pattern them (Heo et al., 2019). Here, we utilize cryoprinting to 3D print low-viscosity materials, such as alginate, and a conducting material, namely poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS), for the fabrication of single or multi-material constructs as a new method for fabricating hydrogel-based electronic devices

Materials and methods

An in-house built cryoplatform, based on a Peltier element, was incorporated into a custom-built bioprinter (Lei et al., 2022). This device is utilized to 3D-print an alginate-based hydrogel as well as PEDOT:PSS, both independently and via multi-material bioprinting for hybrid constructs. Afterward, the electrochemical properties of the constructs were investigated via impedance spectroscopy.

Results and Discussion

First, the temperature of the self-built cryoplatform was characterized by varying the applied voltage. Next, to assess the feasibility of the printing platform, gelatin, which has suitable viscosity properties for 3D printing, was printed onto the platform. After achieving general feasibility of the cryoprinting platform, gelatin was exchanged for the alginate-based hydrogel, which typically cannot be 3D printed in a straightforward manner. The printability of alginate via cryobioprinting is then demonstrated by 3D printing up to 10 layers. To our knowledge, this has not been previously demonstrated for such a low-viscosity material like alginate, without additional crosslinking steps between layers. Subsequently, PEDOT:PSS was cryoprinted and it was investigated how it's electrochemical properties change after cryo exposure. Finally, constructs consisting of both materials were fabricated.

Conclusion

This study demonstrates the feasibility of using cryoprinting towards hydrogel-based electronic devices. We successfully 3D printed a low viscosity alginate hydrogel and a conductive PEDOT:PSS material, both independently and in combination. This technique offers a new approach for creating complex, multi-material constructs without the need for additional crosslinking steps between layers. The ability to cryoprint these materials presents avenues for the advancement of novel biocompatible electronic devices with enhanced functionality.

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COLLAGEN MICROARCHITECTURE DRIVES BREAST CANCER CELL FATE INDEPENDENTLY OF MATRIX STIFFNESS

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Abstract theme: Bioengineered Models / Mechanobigology / Biomaterials / CDT Session

Highly aggressive breast cancer (BC) is the leading cause of cancer related mortality in women globally [1]. In addition to lacking hormonal targets, aggressive BCs display extracellular matrix (ECM) stiffening and abnormal collagen reorganization. These aberrant microenvironments potentiate epithelial-mesenchymal transition (EMT) and invasion [2-3].

To isolate the effects of ECM microarchitecture from mechanics, we developed tuneable collagen fibrillar scaffolds with healthy (porous) and cancer-like (dense) organizations with constant stiffness. Non-malignant (MCF10A) and BC lines (estrogen receptor+ MCF7; triple negative MDA-MB-231) were cultured on these networks for 7 days.

Despite consistent collagen mechanics, BC cells demonstrated enhanced collagen bundling and realignment when interacting with dense (cancer-like) scaffolds, which increased with more invasive phenotypes. Additionally, BC cells acquired aggressive traits including changes to nuclear morphology, enhanced proliferation, and elevated EMT markers. Our data underlines collagen architecture as an independent driver of BC aggression, enabling EMT and invasion.

This novel platform elucidates specific cell-ECM interactions, receptor dynamics, and microenvironment mechanics exploited in cancer progression, unveiling new non-hormonal biomarkers.

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Abstract theme: Mechanobiology

NANOVIBRATIONAL STIMULATION OF MESENCHYMAL STROMAL CELL OSTEOGENESIS – INVESTIGATING THE RELATIONSHIP BETWEEN OSTEOGENESIS, SENESCENCE AND INFLAMMATION

Abstract

Introduction: Bone anomalies have posed a significant social burden on tens of millions of individuals worldwide and a comprehensive understanding of the biomechanics, signs, advancements, and treatments of disorders relating to the bones is becoming a necessity [1]. Conventionally, the field of bone tissue engineering has depended on exogenous agents such as growth factors (e.g., BMP2) and chemicals (e.g. dexamethasone) to regulate and promote regeneration. Nevertheless, limitations, including safety concerns and side effects are making this approach questionable [2]. We have developed a nanovibrational bioreactor that can promote mesenchymal stromal cell (MSC) osteogenesis without the addition of chemical agents [3]. We customized the bioreactor to work at 30 nm amplitude (NK30, 1000 Hz frequency) and preliminary data indicates that MSCs subjected to a higher amplitude of 90 nm demonstrated greater osteogenic differentiation with significant upregulation of RUNX2, osterix, osteonectin, osteopontin, and osteocalcin, but also controlled ROS generation and expression of inflammatory genes, IL-6, TNF-α, and NF-Kb [3]. Considering this data, we aim to investigate the effects of high-amplitude nanostimulation (NK100) on the expression profiles of senescence, inflammation, and osteogenic differentiation markers at both gene and proteome levels and further emphasize respiration-related ROS and inflammation. **Methodology:** To achieve the proposed objectives, we set up the growth conditions for primary bone marrow MSCs as NK30, NK100, osteogenic media (OGM), and a negative control. **Results:** We employed the alamar blue reduction assay and RT-qPCR to inspect the proliferation status of primary bone marrow MSCs grown at each condition for 7, 21 and 28 days and procure the gene expression profiles of relevant senescence, inflammatory, and osteogenic differentiation markers. **Discussion:** Our findings provide a hint that the nanovibrational bioreactor could intensify MSC osteogenesis by reducing senescence and supporting optimal inflammation at a higher amplitude nanostimulation. However, protein-level expression profiling using in-cell western, and ELISA will also be pursued, followed by high-throughput techniques such as flow cytometry for cell cycle and ROS level examination.

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ALTERING CROSS-LINKING DENSITY OF PEG HYDROGELS TO TUNE THEIR VISCOELASTIC PROPERTIES FOR 3D CHONDROGENIC CULTURE OF MSCS

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Introduction. It is known that cells are influenced by the surrounding mechanical properties of their environment. Beyond simply the elasticity of the matrix, its viscoelasticity can have a strong influence on cell behaviour. Viscoelasticity, quantified in terms of the ratio of loss to elastic moduli (*G"/G'* or *tan δ*) or the time taken for a material to dissipate stress under a constant strain, permits cells to remodel their surrounding matrix to facilitate cell adhesions or morphological changes, and effects differentiation lineage.[1] Consequently, controlling the viscoelastic properties of substrates or 3D matrices provides opportunities to enhance tissue culture outcomes in regenerative medicine. For example, regarding chondrogenesis, fast relaxing hydrogels have been shown to facilitate chondrogenic phenotype in 3D bovine chondrocyte culture relative to slow relaxing hydrogels due to the dissipation of mechanical stresses generated by cell volume expansion and ECM deposition.[2] More recently, comparisons of slow and fast stress relaxing collagen hydrogels on chondrogenesis of mouse embryonic stem cells revealed that although slow relaxing hydrogels supported chondrogenesis in short term (one day) culture, these cells succumbed to apoptosis within three days. However, fast relaxing hydrogels mitigated apoptosis and supported long term chondrogenic culture.[3] Here, we have developed a suite of synthetic hydrogels with varying stress relaxing properties using multiarmed polyethylene-glycol (PEG) macromers cross-linked with varying ratios of linear PEG polymer chains. These hydrogels were further biofunctionalised with PEGylated fibronectin to provide motifs for cell adhesion for use in 3D culture of Stro1$^+$-enriched human mesenchymal stromal cells (MSCs) under chondrogenic and growth factor-free conditions.

Material and methods. Fast and slow relaxing hydrogels were prepared using 8-arm 10 kDa (8_{10}) and 8-arm 20 kDa (8_{20}) PEG-maleimide (PEG-MAL) macromers, respectively, cross-linked with 2 kDa (X_2; rigid) or 10 kDa (X_{10}; soft) PEG dithiol (PEG-SH). Fast relaxing hydrogels were cross-linked at 100% efficiency and slow relaxing hydrogels were cross-linked at 80% efficiency. Rheological frequency sweeps (0.1–100 rad·s^{-1}, 1% strain) of the hydrogels to measure their elastic modulus, loss modulus, and *tan δ* were performed at a constant normal force (0.05 N) at 37°C, submerged in PBS solution throughout to prevent dehydration. Axial stress relaxation tests were performed by compressing the hydrogels to 15% strain and measuring the resulting change in stress over 90 minutes. Hydrogels for 3D cell culture included PEGylated fibronectin at 0.5 μg·μL^{-1}. Stro1$^+$ MSCs were cultured in high glucose DMEM supplemented with 10% foetal bovine serum (FBS), Glutamax (1×), non-essential amino acids (1×), sodium pyruvate (1 mM), and penicillin/streptomycin (1%). Cell-laden hydrogels were cultured in DMEM 4% FBS and all other supplements as above. For chondrogenic media, DMEM with 4% FBS, above supplements, and 100 nM dexamethasone, ITS (1×), ascorbic acid (50 μg·mL^{-1}), proline (40 μg·mL^{-1}), and TGF-β3 (10 ng·mL^{-1}). All cell culture performed at 37°C and 5% CO_2. 100% confluent Stro1$^+$-enriched human mesenchymal stromal cells (Stro1$^+$ MSCs) were incorporated into the PEG-SH stock solutions and added the hydrogels at a final seeding density of 5×10^6 cells·mL^{-1}.

Results and discussion. Pairs of stiff ($8_{10}X_2$ and $8_{20}X_2$) and soft ($8_{10}X_{10}$ and $8_{20}X_{10}$) hydrogels with elastic moduli (*G'*) of 5 or 0.4 kPa, respectively, exhibited non-significant swelling differences despite altering viscoelastic properties. Their viscoelastic properties were varied by increasing the PEG molecular weight from 10 to 20 kDa and reducing the cross-linking density to 80% and keeping total PEG concentrations comparable mitigated swelling differences. The *tan δ* increased in both sets of hydrogels, from 0.046 to 0.107 ($8_{10}X_2$ vs $8_{20}X_2$) and 0.065 to 0.556 ($8_{10}X_{10}$ vs $8_{20}X_{10}$). Conversely, decreasing the cross-linking density was detrimental to stress relaxation, yielding lower stress relaxation magnitudes (*ca.* 30% stress dissipated) in comparison to the fully cross-linked hydrogels, which relaxed over 70% of the initial stress inside 90 minutes. Furthermore, gene expression analysis of chondrogenic markers revealed that both the slow and fast relaxing hydrogels can support cell chondrogenesis to at least one week.

Conclusions. Decreasing the cross-linking densities of synthetic PEG hydrogels significantly increased the loss modulus and corresponding *tan δ* of the hydrogels, but lessened the magnitude of stress the gels were able to relax under constant compressive strain. Both sets of hydrogels were supportive of chondrogenic culture, but the hydrogels with greater relaxation potential are expected to support long-term chondrogenesis owing to greater potential of the hydrogels to be remodelled and relax stresses generated during chondrogenesis.

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An Axis of Wnt and Proinflammatory Signals Underlies Mechanically Driven Osteogenesis

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Abstract theme: Mechanobiology

Introduction | Musculoskeletal conditions, affecting approximately 1.71 billion individuals worldwide, stand as the predominant cause of disability globally. Mesenchymal stem cells (MSCs), naturally found in diverse bodily reservoirs, serve as multipotent progenitors for osteoblasts, the cells responsible for bone formation. The process of osteogenic differentiation, leading to the formation of bone, is influenced by a myriad of factors, including the response of cells and the entire organism to biomechanical forces. In our research, we have innovated a nanovibrational bioreactor, a cutting-edge biomechanical approach for directing MSCs towards bone formation. This technology has demonstrated significant efficacy in promoting targeted osteogenesis with high precision in preclinical models. However, the detailed molecular mechanisms underlying this process are yet to be fully elucidated. Our current research explores the impact of nanovibrational stimulation on the osteogenic Wnt signaling pathway, a critical component in bone development.

Materials & Methods | Adipose-derived human MSCs were cultured on our novel bioreactor, delivering nanovibrational stimulation of 30 nm vertical displacement, at 1000 Hz in basal medium ("nano-kicked"; NK) compared to controls stimulated with the osteogenic metabolites (OGM) L-ascorbic acid, β-glycerophosphate and dexamethasone, or controls kept in basal conditions with no stimulus (Ctrl), for 1 to 21 days. Parsing of various pathways involved incorporating Wnt inhibitors, including LGK974, XAV939, AMBMP hydrochloride, CHIR99021, alongside Rock inhibitor Y-27632 and BCL3 mimetic peptide BDP2. Relative gene transcription and protein expression studies were carried out to determine cell molecular responses.

Results | (**A**) An axis of proinflammatory, non-canonical Wnt pathway and osteogenic genes all have higher transcriptional expression in nanovibrated stem cells, compared to metabolite-induced cells. (**B**) Treating nanovibrated cells with Wnt pathway inhibitors XAV939 (targeting canonical-Wnt via Tankyrase) and LGK974 (targeting pan-Wnt secretion via Porcupine) reveals divergent gene transcription signatures – generally, LGK974 reduces expression of genes in the axis, while XAV939 increases axis gene expression. (**C**) In the presence of nanovibration, a BCL3 mimetic peptide (inhibitor of NF-κB signalling, associated with non-canonical Wnt) inverted the transcriptional signatures produced by XAV939 and LGK974 inhibition. (**D**) Further studies inhibiting the Wnt pathway with AMBMP and CHIR99021 recapitulated earlier observations that the non-canonical Wnt pathway is integral to forming the axis of osteogenic differentiation. (**E**) Transcriptional observations throughout were validated with protein expression and use of Rock inhibitor Y-27632 demonstrated functional reversal of nanovibrational signal. (**F**) In complement to hMSCs, BCL3 knockout murine monocytes abrogated the increase in axis genes observed in wild-type cells during nanovibration-driven osteoclastogenesis. While nanovibration itself did not increase osteoclast number or size, XAV939 treatment of BCL3 knockout cells had a dramatic increase in osteoclastogenesis – exemplary of the axis of proinflammatory, non-canonical Wnt in bone cell differentiation.

Discussion & Conclusions | Broadly, a cell signalling axis comprised of non-canonical Wnt signalling, proinflammatory cytokine signalling and NF-κB regulation determines stem cell osteogenic and osteoclastogenic differentiation in response to nanovibrational biomechanical force.

Abstract theme: Mechanobigology /Biomaterials

Remote activation of mechanotransduction via integrin alpha-5 aptamer conjugated magnetic nanoparticles promotes osteogenesis

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Introduction: Magnetic particles (MNP) are used for specific targeting of regions of cell membrane-based receptors by conjugation on the surface with different types of biomolecules. Our research has investigated the use of MNP targeting for applying mechanical force directly to mechano-sensitive regions on the cell receptor thereby stimulating mechanotransduction and downstream signalling [1]. Remote activation of mechanosensors such as ion channels and growth factors has been demonstrated to promote differentiation toward musculoskeletal cell types from multiple stem cell sources used for cell therapies [1,2]. A current challenge of these approaches using antibodies for targeting is non specific binding. In this presentation, we present our work using aptamers as targeting agents. Aptamers are short nucleic acid sequences capable of specific, high-affinity molecular binding that bind to proteins and receptors with a higher affinity compared to antibodies. Aptamers have several advantages, including small size, good biocompatibility, and low immunogenicity [1].
Methods: The MNPs were conjugated with Cd49e antibody for binding to the integrin α5, and CD49e/ CD29 aptamer for binding to the integrin α5β1 receptors. To explore the potential variation in aptamer binding and antibody strategies, antibodies and aptamers were conjugated to MNPs and their signalling efficacy was investigated using Y201 TCF/LEF GFP reporter cells. Aptamer concentration (2.5, 5, and 10 µg/mg) was optimized. Gene expression of RUNX2 and osteopontin, ALP activity, and Alizarin red staining were analyzed.
Results & Discussion: The TCF/LEF reporter cell line demonstrated that mechano-activation of the cells by Fz aptamer MNPs resulted in significant upregulation of the Wnt signaling pathway relative to the activation of the cells by Fz antibody MNPs. Furthermore, the mechano-activation of MSCs by the CD49e/CD29 MNP-aptamers significantly upregulated the expression of the osteogenic marker, RUNX2 compared to the mechano-activation of MSCs by the CD49e MNP-antibodies. In addition, our findings indicate a concentration-dependent response in ALP activity. This trend was consistently reflected in the level of alizarin red staining, as well as the expression levels of osteopontin and RUNX2 (Figure 1). Our results show the responses are upregulated by low concentrations of aptamers ensuring high efficacy with low immunogenicity for osteogenic differentiation for Orthopedic applications.

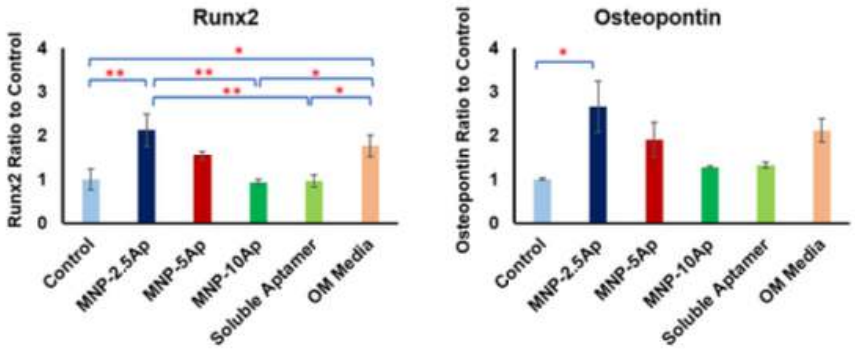

Figure 1. *Quantified data obtained from image analysis of western blot for expression of RUNX2 and osteopontin; Data represented as mean ± SEM (n = 3), and statistically significant differences were marked with * for $p < 0.05$, and ** for $p < 0.01$.*

Conclusion : Our studies provide valuable insights into the use of mechano-targeted MNP platforms for bone regeneration. The study highlights the potential for using aptamer bound platforms for these applications in promoting signaling pathways related to bone formation.
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Abstract theme: Mechanobigology

TOWARDS HIGH THROUGHPUT CELL MECHANOSENSITIVITY ASSAYS

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Introduction

Cell mechanosensitivity is the ability of cells to sense mechanical stimuli arising from their surrounding environment or from within the body [1]. It is inevitably followed by mechanotransduction, the translation of the mechanical stimulus into biochemical signals [2]. Both processes play a crucial role in cells' fate and allow them to adapt to their physical surroundings by remodeling their cytoskeleton, activating different signaling pathways, and changing their gene expression. Thus, they ultimately control physiological processes such as proliferation, differentiation, migration, and apoptosis [3].

Dysregulation of cell mechanosensitivity results in cell dysfunctions and eventually various pathologies, such as cardiovascular diseases [4], osteoporosis [5], intestinal problems [6]. Currently, cellular mechanosensitivity is assessed mainly using single cell biophysical methods. While this approach allows us to characterise the mechanical response of single cells with very high resolution, it offers a very limited throughput. The measure of mechanosensitivity has the expectation to provide physiologically-relevant information, but a paradigm change is required to translate this concept towards biomedical applications, moving to high throughput single cell analysis.

Materials and Methods

Here we propose a concept design of a high throughput mechanosensitivity assay based on the integration of microfluidic and fluorescent microscopy. Floating cells will be aligned within the microfluidic channel and mechanically stimulated due to the channel's geometry. In order to track cells' response, we are using fluorescent ion sensitive dyes and a fluorescent inverted microscope.

Results and Discussion

The findings from this study have the potential to establish mechanosensitivity as a valuable biomarker for the development of advanced label-free cell sorting devices, thereby paving the way for innovative cell therapies. Research into cell mechanosensitivity holds promise in uncovering novel therapeutic approaches for addressing diseases characterized by mechanobiological components.

Acknowledgements

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PEG-BASED VISCOELASTIC HYDROGELS TO INVESTIGATE STEM CELL MECHANOTRANSDUCTION

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Abstract theme: Mechanobiology

Introduction: The microenvironments where cells reside exhibit dynamic and viscoelastic characteristics [1]. These mechanical cues regulate a variety of cellular behaviours, including proliferation, migration and stem cell differentiation, through a process known as mechanotransduction. Our current understanding of these mechanotransductive mechanisms is largely based on *in vitro* studies of the interaction between cells and elastic two-dimensional (2D) substrates, while cells *in vivo* often reside in a viscoelastic three-dimensional (3D) context, where mechanotransductive mechanisms might be different. Indeed, the dynamic viscous behaviour of the environment, coupled with a dimensionality that better mimics the meshwork environment that cells encounter *in vivo*, has been shown to regulate cell fate through mechanisms that, up-to-now, are not completely understood [1]. Hence, here we propose to develop microenvironments, based on 2D and 3D hydrogel systems with controllable viscoelastic properties, to investigate the mechanoresponse of human mesenchymal stem cells (hMSCs), with the aim of exploring the role of dimensionality in the cell mechanotransductive response to viscoelasticity.

Materials and Methods: We prepared non-degradable 3D polyethylene glycol-maleimide (PEG-MAL) hydrogels with diverse viscoelastic properties by varying the molecular weight of the hydrogels' components; 2D PEG-MAL and polyacrylamide hydrogels with the same mechanical properties were used as controls. The hydrogels' mechanics were measured via rheology, atomic force microscopy and nanoindentation. We then utilised immunofluorescence and In-Cell Western techniques to investigate hMSC mechanoresponse and fate within 2D or 3D viscoelastic environments, after functionalisation with fibronectin-derived cell-adhesive RGD.

Results and Discussion: We developed isoelastic PEG-MAL hydrogels with varying viscous behaviour (loss tangent range of 0.05 – 0.25) at different levels of stiffness (soft ~ 1 kPa; rigid ~ 10 kPa), either as 3D cell-laden matrices or 2D substrates; 2D polyacrylamide hydrogels with controlled viscoelastic properties were also developed. After 3 days of cell culture, we found that the hydrogels' viscous character enhanced cell adhesion and mechanotransduction (as measured via nuclear translocation of the mechanosensitive yes-associated protein YAP) on softer hydrogels, while it prevented it on the more rigid ones (Figure 1). This eventually led to changes in hMSC differentiation, whereby osteogenesis was favoured on stiffer elastic hydrogels, while adipogenesis was preferred on softer elastic hydrogels and chondrogenesis on stiffer viscous ones [2]. Similarly, when cells were embedded within 3D matrices, cell spreading behaviour and fate were differentially affected by viscosity depending on the stiffness regime.

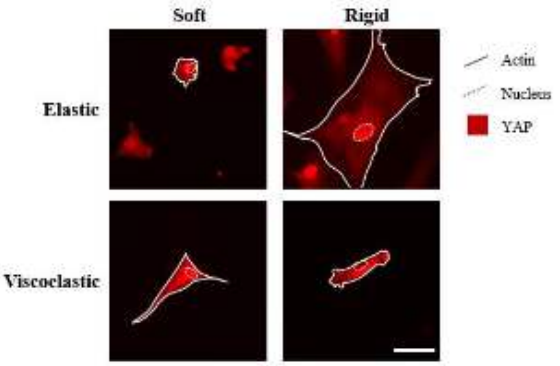

Figure 1. YAP nuclear translocation changes based on the viscoelastic properties of the substrates. Continuous line indicated the cellular outline and dotted line the nuclear outline. Scale bar = 50 µm.

Conclusions: We successfully engineered 2D and 3D hydrogels with tuneable viscoelastic properties in a physiologically relevant range, and we demonstrated their suitability as models to investigate stem cell mechanotransduction. We further demonstrated that viscoelasticity controls the cells' adhesive and mechanotransductive behaviour independently of dimensionality, eventually affecting stem cell fate. The tuneability of our hydrogel models will allow us to investigate the nature and mechanisms of stem cell interactions with viscoelastic substrates in a more *in vivo*-like setting, for example dissecting the interplay between degradability and viscoelasticity in directing stem cell fate.

Acknowledgements
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ABSTRACT TEMPLATE

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Abstract theme: Mechanobigology / Bioengineered Models

The Inhibition of Herpes Simplex Virus Via Cathelicidin LL-37 Peptide: Potential Eye Dropper Delivery Mechanism

Introduction: This project is the first attempt at studying the anti-viral efficacy of LL-37 on HSV-1, both prophylactically and therapeutically when dissolved in an eye dropper medium (SYSTANE® ULTRA Lubricant Eye Drops). Epidemiological studies have reported that over half the world's population has contracted HSV-1 and that infectious keratitis, which may result from this virus, is the primary cause of blindness in the developing world (1). Acyclovir and its prodrug counterparts (Valacyclovir, Ganciclovir, and Trifluridine) are the most common treatment options. However, these drugs have multiple side effects (nausea, headaches, renal cytotoxicity) (2), and have been shown to possess poor uptake into the cornea, low solubility, and an inability to penetrate the tight junctions of the mucin layer and ocular surface epithelium. (3) It is estimated that 23.1% of HSV-1 infected patients receiving prophylactic ACV and Valacyclovir have recurrent infections (4). LL-37 has the potential to address these side effects, and limit recurrent infections.

Materials and Methods: Immortalized HCE-S cells were isolated as distinct epithelial colonies within fibroblasts and infected with HSV-1. To comprehend the rates of proliferation and behaviour of HCE and control (Vero) cell lines, alamarBlue assays were performed and read using the TECAN Infinite® 200-PRO fluorescent microplate reader. Cells were treated with LL-37, dissolved in both PBS and SYSTANE® ULTRA Lubricant Eye Drops, and their prophylactic and therapeutic efficacy was assessed as a measure of (%) cell viability, and HSV-1 gB protein presence.

Results: Though the mechanism in which LL-37 scavenges HSV-1 virions and inhibits HSV-1 replication is unknown, it was found that LL-37 can prophylactically inhibit HSV-1 propagation when dissolved in PBS in HCE-S cells cultured in Keratinocyte serum-free media (KSFM). In the HCE: KSFM experimental condition, prophylactic LL-37 in PBS 20µg/mL exhibited a 2.49-fold increase in (%) cell viability at 20µg/mL relative to the baseline. Cells treated with the same concentration of LL-37 also showed a noticeable absence of HSV-1 gB protein signal through immunocytochemistry. This therapeutic potential was not exhibited in LL-37 delivered in SYSTANE® ULTRA Lubricant Eye Drops (maximum increase in (%) cell viability= 1.39-fold increase at 10µg/mL).

Discussion: LL-37 imparts a lower toxicity, a high solubility, and greater biomimetics when compared to Acyclovir and its analogs. Though the chemotactic mechanism of this AMP is not completely understood, it is suspected that the amphiphilic properties are what allow it to infiltrate the tight junctions of the epithelium, while its cationic nature can deregulate the negatively charged genome of HSV-1 (5).

Conclusions: This increase in (%) cell viability resulting from PBS-dissolved LL-37, the qualitative decrease in HSV-1 gB glycoproteins in PBS-dissolved LL-37 treated wells relative to controls, and the lack of cytotoxicity exhibited across all PBS-dissolved LL-37 peptide concentrations contribute to the pharmaceutical translational potential of this antimicrobial peptide.

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